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Vol. 36 September, 1959 No. 5

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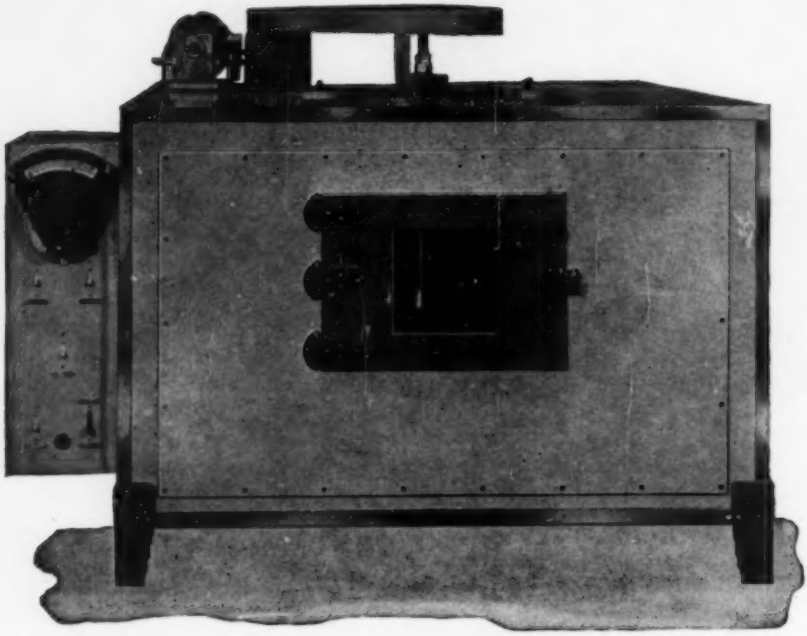

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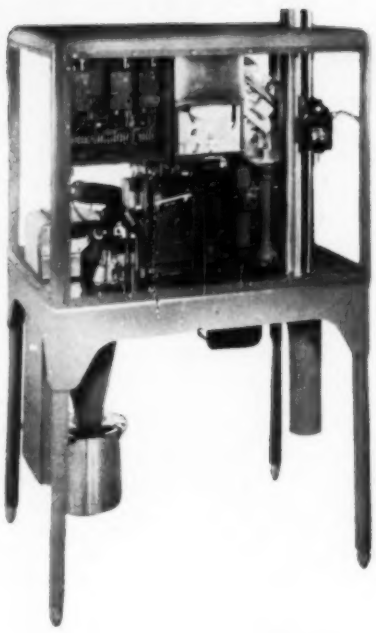

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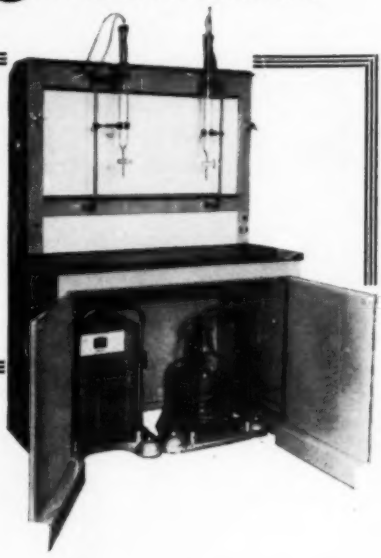

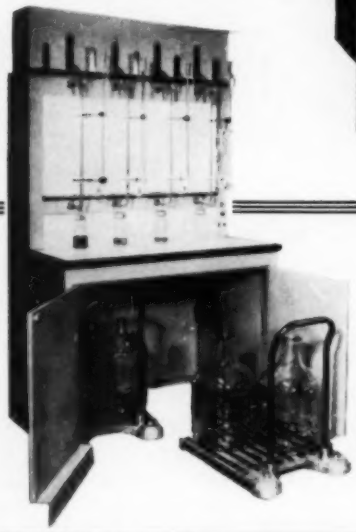

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CEREAL CHEMISTRY

VOL. 36 SEPTEMBER, 1959 NO. 5

A NEW GRAIN HARDNESS TESTER[1]

R. KATZ, A. B. CARDWELL, N. D. COLLINS,[2] and A. E. HOSTETTER[3]

ABSTRACT

A hardness tester, especially adapted for grain, was constructed by modifying a commercial portable soft metal tester known as the Barcol Impressor. A preloaded stylus is forced into grain sections prepared by means of a microtome. The displacement of the stylus, measured by a dial micrometer, is used as a hardness index. This has been related to the Vickers diamond pyramid hardness which is a standard metallurgical test. A number of hardness measurements may be made on a single transverse kernel section. The tester may be used for all wheat varieties. Significant variations in hardness within a single wheat kernel section have been demonstrated. While the hardness of a block of lead varied over ±½ hardness number, a kernel section of hard winter (Ponca) wheat exhibited hardness numbers ranging from 25 to 40, on the arbitrary scale of this tester.

Such terms as *hardness* and *vitreousness* have long been used to describe properties of grain of interest to wheat breeders and millers. Biting or cutting grain has provided a qualitative evaluation of this property. A number of attempts have been made to find a quantitative measure of the hardness of individual kernels or of the average hardness of a collection of kernels.

The Smetar hardness tester, produced by Miag, utilizes the penetration of a diamond-shaped stylus into a section of a kernel as a measure of hardness (4). The impression in the endosperm is measured with a microscope. This apparatus is similar in conception to the Vickers hardness tester used in metallurgy, except that the stylus of the Smetar tester is larger. That is necessary, for the impression made in a wheat kernel section by a Vickers tester is difficult to measure with the microscope attached to the Vickers machine. As a result of the size of its stylus, essentially only one or two measurements can be made on a wheat kernel section with the Smetar tester.

A traditional method for measuring the hardness of bulk samples

[1] Manuscript received October 27, 1958. Contribution No. 69, Department of Physics, Kansas Agricultural Experiment Station, Manhattan.
[2] Department of Physics.
[3] Department of Industrial Engineering.

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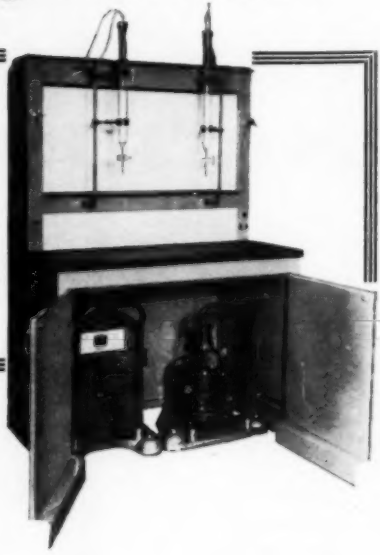

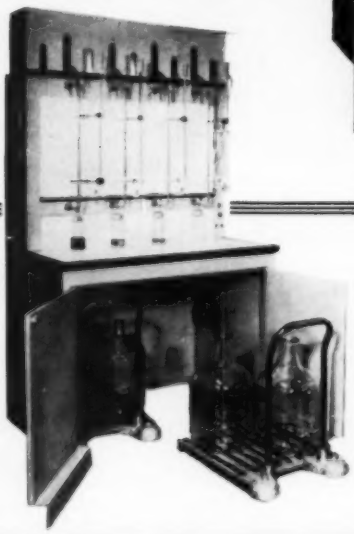

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Such terms as *hardness* and *vitreousness* have long been used to describe properties of grain of interest to wheat breeders and millers. Biting or cutting grain has provided a qualitative evaluation of this property. A number of attempts have been made to find a quantitative measure of the hardness of individual kernels or of the average hardness of a collection of kernels.

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is the laboratory-scale barley pearler (2). The fraction of a small sample of wheat which is pearled off in a short interval by an abrasive stone is used as a hardness index.

Another index of hardness which has been used is the energy required to mill 1 g. of grain. The Brabender hardness tester, which consists essentially of a small burr mill fitted to the dynamometer coupling of the Farinograph, has been used by Paukner (3). Basically this device measures the torque required to drive the burr mill as a small sample of grain is milled. The data are then reduced to terms of the energy required per milled gram of grain.

In the present work, the penetration of a small spring-loaded stylus into a kernel section is used as the basis of hardness measurement. A commercial portable hardness tester called the Barcol Impressor (manufactured by the Barber-Colman Co., Rockford, Illinois) has been adapted for work with grain by suitable choice of the stylus tip and of the spring loading. It has been possible to produce one model of the tester which is capable of 30 or more independent hardness measurements on a single wheat kernel section of hard wheat varieties. By increasing the radius of the stylus tip a second, more versatile model of the tester has been developed which is applicable to all wheat varieties under a wide range of ambient humidity. Approximately six independent measurements of the hardness of a single transverse kernel section may be made.

Conversion of the Barcol Impressor into a Hardness Tester Suitable for Testing Wheat Sections

The Barcol Impressor is a portable hardness tester designed for testing soft metals. The tester consists essentially of a spring-loaded stylus, a case, and a dial micrometer. Hardness is measured by the distance the stylus is displaced into the case when the tester is pressed against a test object. The greater the displacement of the stylus, the harder the object. Readings of the micrometer are referred to as hardness numbers. Adjustments are provided for setting the initial compression of the spring, and for setting the reading of the micrometer at its greatest deflection. The dial micrometer provided with the instrument has 100 divisions.

For work with wheat, several modifications of the Impressor were necessary. To provide a means of mounting and positioning a wheat kernel, the Impressor and a two-way microscope stage were mounted on an oak platform. Kernel sections, cemented to microscope slides, could then be set into place and located to 0.1 mm. by the Vernier scale of the microscope stage. A photograph of the modified Impressor

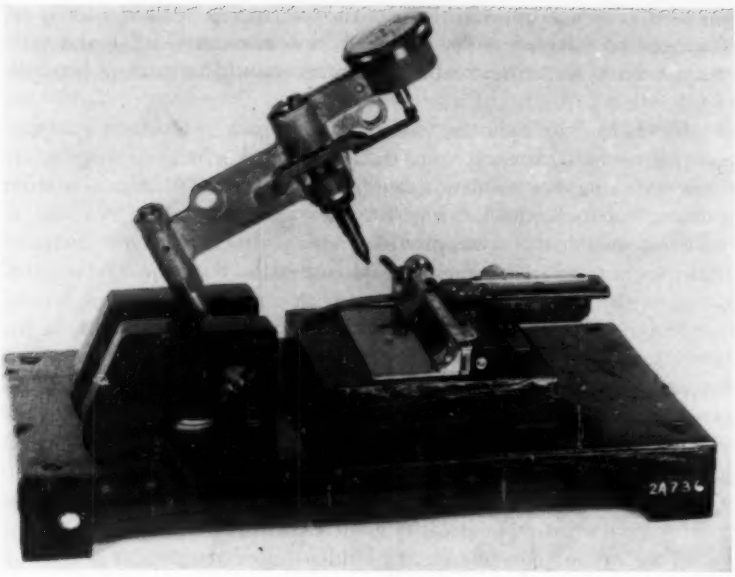

Fig. 1. The converted Barcol Impressor.

is shown in Fig. 1, and a sectional line drawing is shown in Fig. 2.

In Model I of the hardness tester, used to explore the point-to-point variation of a single kernel, a stylus having a conical tip with 25° included angle and terminated in a spherical surface of radius 0.0048 in.

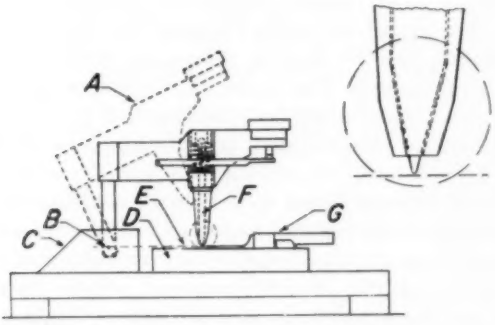

Fig. 2. Diagram of the converted Barcol Impressor. A, tester in lifted position; B, axis about which the tester swings; C, oak supports; D, oak platform which supports the sections to be tested; E, plane which contains the upper surface of the sections and the axis mentioned in B; F, penetrator; G, two-way movement stage.

was used. The maximum reading of the micrometer scale was set at 50. The applied force necessary to achieve a maximum reading was 2 lb. Measurements on hard wheat kernel sections could be made at intervals of 0.4 mm.

Model II of the hardness tester was developed in an effort to obtain a device suitable for comparing the hardness of all wheat varieties under a wide range of ambient humidity conditions. This was of similar construction to Model I except for the stylus and spring. A stylus of hardened steel drill rod was provided with a conical tip of 13° included angle, terminated in a spherical surface of radius 0.011 in. The original spring of the Impressor was replaced with a music wire spring having free length 1.25 in., wire diameter 1/32 in., outer diameter 21/64 in., eleven free coils, and plain ground ends. It was necessary to use different compressions of the spring for different wheats, because soft wheat varieties crumbled under test conditions suitable for hard wheats. The dial was adjusted to read 100 for maximum deflection of the stylus. A force of 2.2 lb. was necessary to achieve maximum deflection at the soft wheat compression and 3.6 lb. at the hard wheat compression. The compression adjustment is easily made by turning a slotted nut at the top of the instrument (see Fig. 1). Under these circumstances the hardness of a pure lead block was read as 93.5 on the soft wheat scale and 81.5 on the hard wheat scale.

Procedure

Sections of wheat kernels were prepared with a freezing microtome, following the technique of Grosh and Milner (1). Kernels were placed on the microtome stage and embedded in warm aqueous gelatin solution. Freezing was quickly accomplished with intermittent blasts of carbon dioxide. The kernel was pared down to the desired position and removed from the stage by thawing the gelatin with a soldering iron applied to the stage itself. The kernel slab was then inverted on the stage and refrozen. The sections were then cut to a thickness of 0.8 mm. by cutting the grain to the thickness of a piece of balsa wood frozen to the stage. For good hardness readings, the kernel section must have parallel faces. Transverse kernel sections were used to avoid difficulty with the crease of the kernel in subsequent hardness measurements.

Immediately after the kernel section was prepared, it was cemented to a glass microscope slide with Duco cement. After the cement had dried the kernel was exposed to appropriate experimental conditions (for example, in a humidity chamber) and its hardness tested.

In testing, the microscope slide was mounted on the microscope stage of the hardness tester. Readings from the microscope stage al-

lowed the position of the measurement to be plotted on an enlarged diagram of the kernel to within ±0.05 mm. The actual hardness measurement was made by pressing down on the framework of the Impressor with the hand until the flat part of the Impressor spindle was in contact with the specimen. The dial reading reached a maximum value and remained there as long as the force was applied. This reading was recorded as the hardness number of a particular point on the wheat kernel. The results were not altered by variations in hand pressure. About 50 kernels could be sectioned and measured at about six different points on each section in the course of a working day.

Testing Accuracy of the Modified Instrument

To test the accuracy and reproducibility of the readings of the modified Barcol Impressor, a series of blocks of lead alloys was prepared. The lead blocks were made with antimony content ranging from 0 to

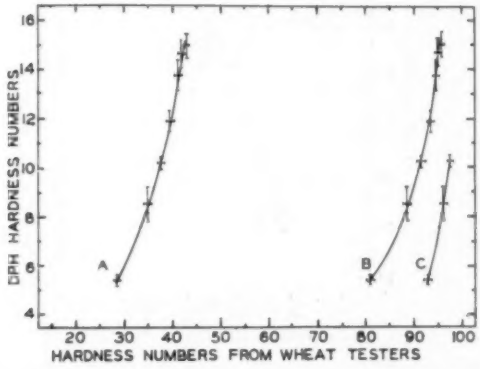

Fig. 3. Diamond Pyramid Hardness, as found with a Vickers hardness tester on samples of lead-antimony alloy, vs. hardness values from the two models of wheat testers found on the same samples. The samples ranged from 0 to 6% antimony. Curve A concerns model I; curve B concerns the hard wheat range of model II; curve C concerns the soft wheat range of model II.

6%, in steps of 1%. A series of nine hardness measurements on each block was made with the two models of this tester, and with the Vickers tester. The results are shown in Fig.3, where each point is plotted as a cross giving the mean hardness and the standard deviation of each block. From the diagram it is apparent that the consistency of the wheat hardness testers is within ½ hardness number for a single specimen. Some variations may be due to nonuniformity of the specimen, as may be inferred from the range of DPH numbers for each point.

Measurements of the hardness of wheat resulting in greater variation than ½ hardness number must be attributed to hardness differences in the wheat kernel itself.

To relate the two scales of Model II of the wheat hardness tester, a series of measurements of durum (Mindum) wheat kernels was made. Durum wheat kernels had shown the greatest uniformity of all wheat tested. Groups of 16 durum wheat sections were exposed to varying conditions of humidity. After the sections had come to equilibrium, one measurement was made on each cheek of each section with the soft wheat range, and one measurement on each cheek with the hard wheat

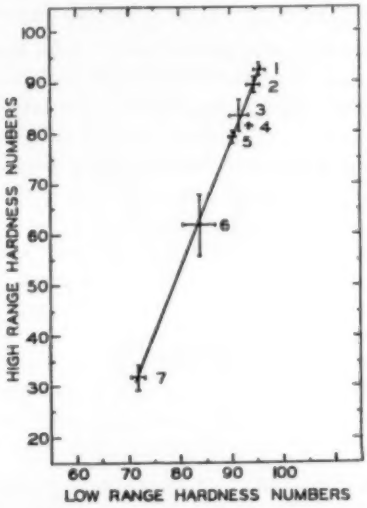

Fig. 4. Graph relating hardness values from the hard wheat (high) range of model II to values from the soft wheat (low) range. The samples used are: 1, durum sections exposed until an equlibrium condition was reached at 33% relative humidity (r.h.); 2, durum sections from wheat at 75% r.h.; 3, durum sections from wheat at 81% r.h.; 4, pure lead; 5, indium solder; 6, durum sections from a 93% r.h.; 7, polyethylene.

range. The 32 hardness values thus obtained for each humidity were then averaged. The results of these measurements are shown in Fig. 4. Measurements with the two ranges were also made on blocks of pure lead, indium solder, and polyethylene. Points obtained from these measurements are also indicated in Fig. 4.

Results

Figure 5 illustrates the data which can be obtained with Model I of this hardness tester. The hardness of three different sections taken from

three different kernels of hard red winter wheat (Ponca) is shown. These sections were taken from the germ end, the center section, and the brush end of their respective kernels. The size of the impression

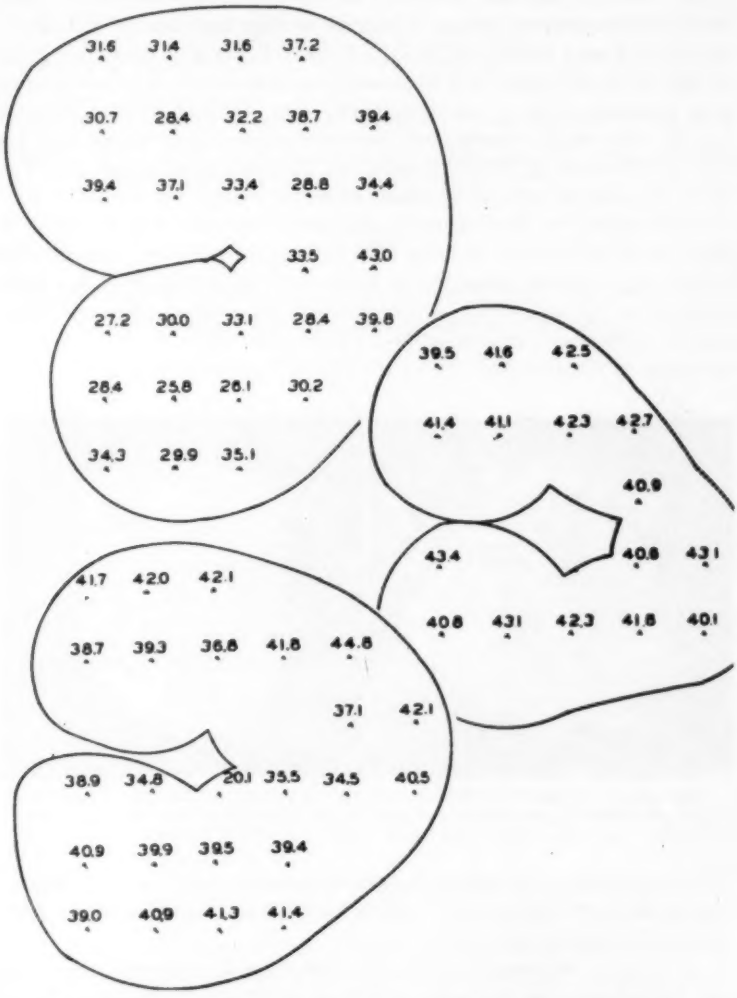

Fig. 5. Hardness values across sections of Ponca wheat, made with model I of the hardness tester. Top, section from germ end; bottom, middle of kernel; right, brush end.

made by the stylus is about the smallest circle which can be drawn around the hardness number.

While these data cannot be considered as representative of the particular variety, several interesting observations can be made. In each of the three sections the periphery of the kernel appears harder than the region around the crease. Thus the average hardness of the center section of Ponca wheat was 39.7 ± 2.5 while the ring of readings closer to the crease averaged 34.9±0.4, and the ring of values closest to the bran averaged 41.6±1.7. Similar results were obtained with sections of durum (Mindum) wheat. A brush section of durum wheat had an average hardness of 38.9±2.2 while the hardness of its inner ring was 36.8±2.1, and the average hardness of its outer ring was 40.0±2.0. The numbers following the ± sign are standard deviations. The hardness of these hard winter and durum sections was comparable, though the durum sections were more nearly uniform. A variation of 10 hardness numbers across one Ponca section was not uncommon, while the hardness of a Mindum section seldom varied more than 4 or 5 hardness numbers.

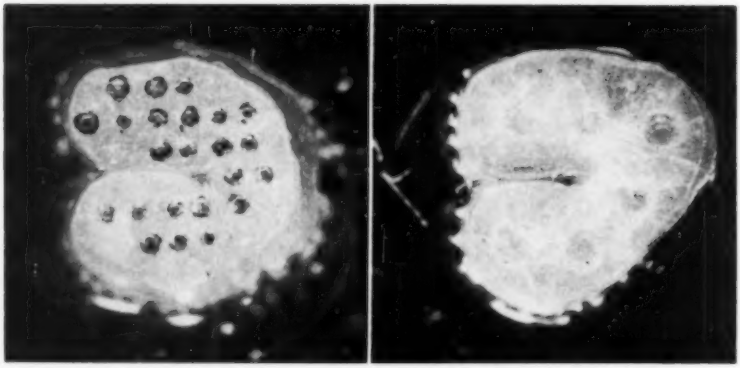

Fig. 6. Left, durum after testing with model I. So that the indentations could be more evident, the penetrator was dabbed with ink before each test. Right, photo of durum after testing with model II.

The photographs of kernel sections after hardness testing with the two models of tester in Fig. 6 show that there is no interference between adjacent hardness measurements.

A number of points will bear further investigation. Among these are the manner of specimen preparation, that is: the influence of freezing, thawing, and cementing on hardness measurements; the influence of ambient humidity and of varietal and agronomic conditions

on wheat hardness. The present paper describes an apparatus which has been developed for wheat hardness measurement and a few specimen results illustrating its use.

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TREATMENT OF WHEAT WITH IONIZING RADIATIONS
IV. Oxidative, Physical, and Biochemical Changes[1]

SING-PING LAI, K. F. FINNEY, AND MAX MILNER[2]

ABSTRACT

Gamma irradiation in dosages up to 1 million rep was applied to a hard red spring wheat (Conley) and a hard red winter wheat (Comanche). Drastic damage to the starch fraction of flours milled from these wheats was indicated by a decrease in gelatinization viscosity and starch content and an increase in maltose value in a dosage range of 100,000–1,000,000 rep. Protein hydration capacity decreased. Carotenoid pigment content of the flour was reduced, yet flour color was darker, as indicated by reflectance. An increase in fluorescence at the highest dosage suggested that darkening resulted from stimulating a browning reaction.

Baking trials with various test formulas (100 g. flour) indicated progressive damage to loaf volume and crumb grain, texture, and color with increasing irradiation. Potassium bromate effectively reversed decreases in loaf volume due to irradiation, but crumb grain was not improved by this oxidizing reagent, indicating a departure from the classic bromate effect. Irradiation produced fermentable degradation products of starch which caused moderate increases in loaf volume in the absence of sugar and malt in the baking formula. However, optimum baking performance could be obtained only with the addition of sugar and malt. Crumb firming on storage was increased by irradiation, despite drastic changes in the starch fraction. Bread flavor decreased linearly with increasing radiation damage.

The promising potentials of food irradiation for preservation and other useful effects already has stimulated the beginning of an exten-

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sive literature concerning wheat and flour irradiation (4,5,9,10,12,13, 15,16,17,18).

Previous work in this laboratory has shown that the respiratory pattern reflecting the growth of grain-spoilage fungi can be eliminated from hard red winter wheat at moisture levels of 12 and 20% by gamma irradiation treatment as low as 250,000 rep (17). Seed germination is readily destroyed, particularly when the grain is damp. No hydrolysis of fat occurs even with megarep dosages, although browning phenomena (as indicated by increased fluorescence values) are accentuated at very high levels of treatment. This work also showed that radiation sharply reduces gelatinization viscosity of starch fractions and significantly modifies physical properties of doughs (16). However, since this earlier work presented only preliminary information concerning the effects of irradiation on baking properties, this study was undertaken to evaluate such factors more critically and to clarify other biochemical effects observed previously.

Materials and Methods

Two varieties and classes of wheat were studied. Conley, a hard red spring wheat obtained from North Dakota, contained 9.7% moisture and 14.4% protein. Comanche, a hard red winter wheat harvested at Kansas State Agronomy Farm, Manhattan, contained 11.9% moisture and 14.2% protein. These wheats were sealed in no. 2 tin cans and sent to the Material Testing Reactor, Idaho Falls, Idaho, for radiation treatment at levels shown below:

Radiation Dosage $\times 10^6$ *rep*	*Exposure Time*[a]	
	Conley	*Comanche*
	min:sec	*min:sec*
Control	0:00	0:00
0.1	1:52	2:42
0.3	5:31	8:00
0.6	11:24	16:00
1.0	18:22	27:00

[a] Rates of 3.27 and 2.24 $\times 10^6$ rep/hour for Conley and Comanche, respectively.

After their return, the irradiated cans of wheat were opened, and each treatment group was mixed 30 minutes. The wheat was tempered to 15% moisture and milled on a Buhler experimental mill. The flour was mixed 30 minutes and stored at room temperature for further study.

Analytical Methods. Moisture, protein, ash, reducing sugar, nonreducing sugar, fatty acid, pigment content, maltose value, sedimenta-

tion value, farinograph tests, and amylograph values of flours were determined according to *Cereal Laboratory Methods* (1,2). Starch content by polarimetric procedure was determined according to AOAC method (3). Fluorescence determination was based on the Cole and Milner method (6).

The method of Miller *et al.* (14) was employed to determine the quantity of dextrin in flour and average dextrin chain length of the starch. For this determination, a 50-g. flour sample was extracted with 250 ml. of water at 30°C. The water extract was autoclaved 20 minutes to destroy residual enzyme activity and then fermented 18 hours with baker's yeast (*Saccharomyces cerevisiae*) to eliminate fermentable sugar. The dextrin was precipitated by adding 95% ethanol (extract: ethanol ratio 1:3 by volume). One aliquot of the precipitated dextrin was filtered through a Gooch crucible and weighed after 2 hours' drying at 130°C. Another portion of the precipitate was redissolved in water to determine reducing groups both before and after acid hydrolysis with 0.5*N* sulfuric acid. Autoclaving was at 15 lb. pressure for 30 minutes. Dextrin chain length was calculated by dividing the number of reducing groups determined after hydrolysis by the number before hydrolysis.

The Kent-Jones flour color grader was used to measure flour color (11). This instrument utilizes a balanced circuit containing two photoelectric cells to measure light of a specific wave length reflected from the surface of a paste prepared from 30 g. of flour and 50 ml. of water. The amount of light reflected by the flour depends on the quantity and nature of the bran powder but not on the granularity of the sample.

Baking Procedure. Baking tests were carried out with a straight dough method using 100 g. flour, as developed by Finney and Barmore (7,8) with four different formulas: 1), a rich formula including flour, milk, shortening, sugar, salt, malt, yeast, water, and potassium bromate as needed; 2), the same formula without malt; 3), the same formula without sugar; 4), the same formula without sugar and malt. Modifications in formulas 2, 3, and 4 were made to clarify effects on baking properties of starch modification due to radiation.

Commercial pound loaves were baked by a sponge-dough method that included flour, yeast food (Arkady), malt, yeast, water, sugar, salt, nonfat dry milk, and shortening.

Compressibility of bread crumb was expressed as weight required to depress a 1-in. plunger 4 mm. into two ½-in. slices of commercial pound loaves by a Bloom Gelometer. Three determinations on two of four slices of bread from each experimental group cut by an Oliver

bread slicer were recorded 2 and 24 hours after baking.

One-pound regular commercial loaves produced from flours milled from irradiated Comanche wheat of various dosage levels were examined for taste by seven judges on the day following baking. When cool, the freshly baked loaves were wrapped in waxed paper and stored at room temperature until used. The form used by the judges follows:

Date_____

Signed_____

Flavor rating

Best_____

2nd best_____

3rd best_____

4th best_____

5th best_____

Please rate *each of the five according* to **FLAVOR** preference.

All samples were coded in a random fashion, a new code being used for each day. Six panel tests were carried out every other day.

Results

Chemical and Physical Properties of Flour Milled from Irradiated Conley Wheat. Table 1 indicates the chemical and physicochemical properties of flours milled from Conley wheats treated with various irradiation dosages.

Slight increases in reducing and nonreducing sugar content of flour were found. Maltose value also increased. As radiation dosage increased, maximum viscosity of starch gelatinization decreased considerably. The quantity of dextrin with short average glucose chain length was higher in treated flour than in untreated flour. A tendency toward decreased starch content appeared with irradiation, particularly at the 1-megarep treatment level.

No important change in fatty acid content was detected. Fluorescence was unchanged in the lower dosage treatment but showed a slight increase at the 1-megarep treatment, indicating that this high irradiation level caused browning.

A loss of swelling properties of flour gluten was shown by the decrease in sedimentation value of flour milled from irradiated wheat.

Flour color as revealed by the Kent-Jones color grader was consistently darker with increasing dosage level. Nevertheless, the more highly treated flour had a lower carotenoid pigment content.

Farinograph data revealed increased water absorption, decreased

TABLE I

CHEMICAL AND PHYSICAL PROPERTIES OF FLOURS MILLED FROM IRRADIATED CONLEY WHEAT

DETERMINATION	RADIATION DOSAGE × 10^6 REP				
	Control	0.1	0.3	0.6	1.0
Protein, %	12.9	13.1	12.9	13.0	12.9
Moisture, %	11.5	11.6	11.6	11.9	11.7
Ash, %	0.43	0.43	0.43	0.43	0.43
Reducing sugar[a]	173.0	184.4	184.4	184.4	190.8
Nonreducing sugar[b]	272.5	282.5	282.5	282.5	287.5
Maltose value[c]	454.5	461.5	499.0	542.0	550.0
Dextrin[d]	1048.4	1374.6	1639.6	1655.9	1678.1
Dextrin reducing matter[e]					
Before acid hydrolysis	32.8	80.3	141.2	152.7	171.3
After acid hydrolysis	368.5	390.2	531.5	595.3	654.1
Glucose units in dextrin	12	5	4	4	4
Fatty acids	13.2	12.9	12.6	12.3	12.3
Fluorescence value	27	28	28	29	31
Pigment content[f]	13.7	12.9	12.4	11.6	10.5
Starch, %	70.1	68.7	68.6	67.5	62.3
Sedimentation value	66.0	57.0	53.0	51.0	47.5
Flour color	2.77	3.40	3.46	3.69	3.95
Maximum amylograph viscosity[g]	950	650	310	200	90
Farinograph data					
Absorption, %	72.8	73.5	74.4	75.0	75.1
Dough devel. time, minutes	8¾	8	7	6	6½
Tolerance index[g]	25	30	25	35	45

[a] Mg. maltose per 10 g. flour.
[b] Mg. sucrose per 10 g. flour.
[c] Mg. maltose per 10 g. flour per hour at 30°C.
[d] Mg. per 100 g. flour.
[e] Mg. maltose per g. dextrin.
[f] Mg. carotene per 100 g. flour.
[g] B.u.

dough development time, and a general increase in mixing tolerance.

Baking Properties of Flour Milled from Irradiated Conley Wheat. Table II compares the baking quality of wheat flour after various irradiation treatments. Figure 1 is a photograph of the loaves obtained from these tests.

Baking absorption increased somewhat up to 0.6×10^6 rep but at the maximum dosage of 1.0×10^6 rep was slightly less than the control. Mixing time decreased consistently. Bread baked with the rich formula at zero bromate level showed a progressive reducing effect with increasing radiation dosage in terms of lower loaf volume and poorer (underdeveloped) crumb grain. Crumb grains were progressively poorer with increasing radiation dosage. For the three highest dosages, consistent increases in dark color and musty flavor were noted. However, with the addition of adequate quantities of potassium bromate, the loaf volumes for all treatments were fully equal to that of the control. It is pertinent, however, that adding optimum levels of bromate, which produced a regain in loaf volume, did not result

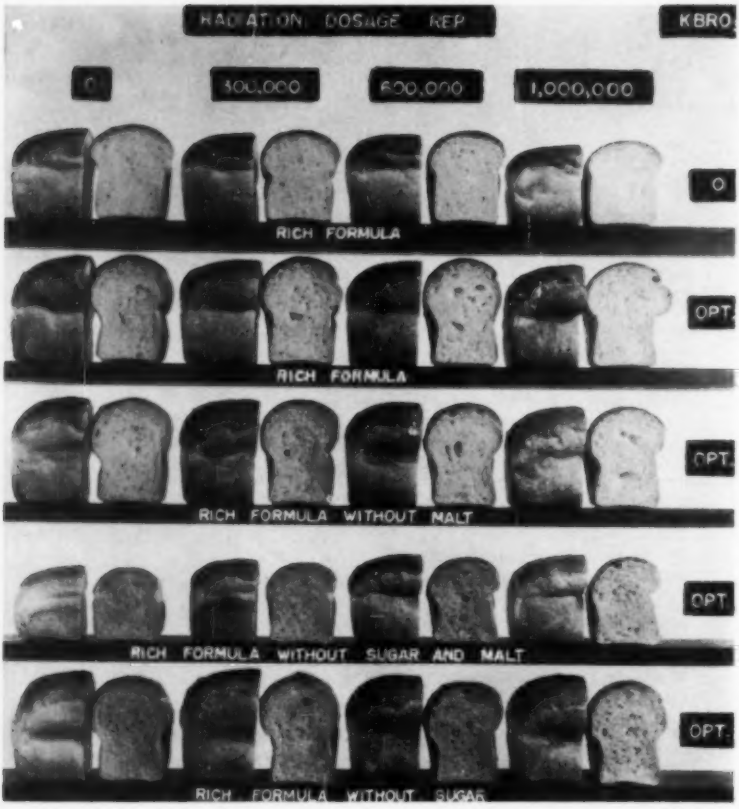

Fig. 1. Loaves produced by different formulas from gamma-irradiated Conley wheat.

in recovery of crumb grain quality equal to that of the control. An increase in optimum potassium bromate requirement was noted as radiation treatment increased. Thus, optimum loaf volume and crumb grain structure were obtained by adding 4 mg. potassium bromate for the 1-megarep treatment compared with only 2 mg. for the control. Loaf volume differed only slightly, in general, between different treatment levels within each of formulas 2 and 3 (without malt and without sugar, respectively). Crumb grain, however, was progressively poorer with increasing radiation dosage. Bread produced by formula 4 (without sugar and malt) showed a regular increase in volume up through a dosage level of 0.6×10^6 rep but dropped again at 1.0×10^6 rep.

TABLE II

BAKING CHARACTERISTICS OF FLOURS FROM IRRADIATED CONLEY WHEAT

Radiation Dosage × 10^5 Rep	Absorption	Mixing Time	Optimum Bromate	Loaf Volume		Crumb Grain [a]	
				0 Bromate	Optimum Bromate	0 Bromate	Optimum Bromate
	%	*min*	*mg/100 g*	*cc*	*cc*		
			Rich formula				
Control	74.5	4¾	2	840	941	S	S
0.1	74.8	4¼	2–3	805	946	Q	S
0.3	74.9	3⅝	3	805	965	Q–U	Q–S
0.6	76.0	3	3	763	969	U	Q–U
1.0	74.0	2⅜	4	704	945	VU	U
			Rich formula, without malt				
Control	76.5	5⅛	1–2	...	935	....	S
0.3	77.3	3⅞	2–3	...	943	....	Q–S
0.6	78.0	3	3	...	946	....	Q
1.0	76.0	2⅜	4	...	914	....	Q–U
			Rich formula, without sugar				
Control	76.3	5	1–2	...	940	....	S
0.3	77.1	3⅞	2–3	...	963	....	Q
0.6	77.8	3	3	...	973	....	Q–U
1.0	75.8	2⅜	3–4	...	947	....	U
			Rich formula, without sugar and malt				
Control	77.8	4¾	1–2	...	568	....	Q–S
0.3	78.6	3⅝	2–3	...	677	....	Q
0.6	79.3	3	3–4	...	735	....	U
1.0	77.3	2⅜	4	...	706	....	U

[a] S, satisfactory; Q, questionable; U, unsatisfactory; VU, very unsatisfactory.

The Taste Panel Test. One of the seven judges was absent during the last two of the six tests. A summary of the scores appears in Table III. An analysis of variance and of the linear component of these data showed that both F values are significant at the 0.5% level.

TABLE III

TASTE SCORES OF BREAD BAKED FROM IRRADIATED COMANCHE WHEAT

Radiation Dosage × 10^5 Rep	Average Score of Replication [a]						Sum of Average Taste Scores	Mean Average Taste Scores [b]
	I	II	III	IV	V	VI		
Control	2.85	1.57	1.28	1.00	2.00	1.83	10.53	1.76
0.1	2.43	2.43	2.43	2.71	2.16	2.50	14.66	2.44
0.3	2.28	3.43	2.57	3.00	2.50	1.83	15.61	2.60
0.6	2.71	3.57	3.85	3.28	3.83	3.83	21.07	3.51
1.0	4.71	4.00	4.85	5.00	4.50	5.00	28.06	4.68

[a] Results of seven judges. Score of best is 1, second best is 2, etc.

[b] F values for analysis of variance and linear component were significant at the 0.5% level.

TABLE IV

EFFECT OF GAMMA-IRRADIATION ON CHANGES IN RESISTANCE TO COMPRESSION (STALING) OF BREAD BAKED FROM FLOURS REPRESENTING DIFFERENT DOSAGE LEVELS

Radiation Dosage × 10^6 Rep	Group Means[a]		Difference	Average Staling Rate[b]
	24 Hour	2 Hour		
	g	*g*	*g*	*g/hour*
Control	64.6	39.4	25.2	1.15
0.1	73.1	42.0	31.1	1.41
0.3	77.2	48.9	28.3	1.29
0.6	89.7	50.2	39.5	1.80
1.0	115.4	68.6	46.8	2.13

[a] The mean value of three determinations on two of four slices of bread from each of the four different daily samples.
[b] Difference between 24- and 2-hour group means divided by the time difference.

Thus, there is a highly significant decrease in desirability of the taste and flavor of bread due to irradiation. This decrease is in proportion to the increase in radiation dosage.

Crumb Compressibility of Bread Baked from Irradiated Comanche Wheat. Firming of crumb, computed as increase in resistance to compression, increased with increasing dosage level, except for an anomalous value at the 0.3×10^6 rep treatment (Table IV). F values for the analysis of variance and linear component of the 2-hour resistance-to-compression values were significant at the 0.5% level.

Discussion and Conclusion

Starch damage due to irradiation was reflected by a number of factors including a decrease in actual starch content at high irradiation level, a drop in gelatinization viscosity, an increase in dextrins and a decrease in their chain length, and an increase in reducing sugars. This major degradation of starch suggests that the polysaccharides of wheat are the fractions most drastically affected by irradiation.

Increases in absorption with increasing dosage up to 0.6 megarep, as shown by both farinograph and baking tests, also can be attributed to starch damage by radiation. At the highest dosage (1.0 megarep), the absorption decreased, apparently because of an effect of radiation on some other constituents of wheat.

Fatty acid content is unchanged by irradiation, indicating that radiation does not produce fat hydrolysis. Fluorescence increased slightly at the 1-megarep dosage level, confirming the findings of Yen *et al.* (18) that heavy irradiation dosages initiate a browning reaction in wheat. This is further confirmed by the observed loss in carotenoid pigment content of flour that occurs with irradiation even as the treated flour (as revealed by the Kent-Jones color grader) becomes darker owing to

a browning reaction. As indicated by the work of Yen *et al.* (18), a considerable increase in fluorescence occurred in grain at a dosage level of 1,875,000 rep. McWilliams *et al.* (12) also observed darkening in bread baked from irradiated flour.

Loss of gluten hydration capacity occurred as indicated by a decrease in sedimentation value; however, the modification in protein properties due to irradiation appears to be nominal compared with effects on the starch fraction.

The questionable-to-unsatisfactory crumb grain in bread obtained from irradiated wheat when bromate was omitted from the formula may be emphasized. An increasing reducing effect with increasing dosage is indicated. With quantities of bromate adequate for complete loaf volume recovery, however, the questionable-to-unsatisfactory crumb grains with open, heavy, and contrastable cell structure suggest impairment and overoxidation. Thus, the apparent reducing effect of radiation in terms of decreased loaf volumes and underdeveloped crumb grain which is most obvious at the zero bromate level does not follow the generally accepted concept of the effects of reduction on bread flours. The apparently underdeveloped crumb grains do not recover, along with loaf volume, to equal the characteristics of the control when bromate is added, which is the normal response to this reagent. Thus, besides a reducing effect, radiation adversely and irreversibly affects the flour in a manner that differs from the conventional reversible reduction-oxidation concept.

The peculiar response to potassium bromate of flour milled from irradiated wheat suggests that compounds other than protein and starch are involved. It is conceivable, for example, that pentosans, being complex polysaccharides similar to starch, may also be seriously altered by radiation. Flour contains approximately 1% of water-soluble pentosans which have been shown to form irreversible gels on treatment with oxidizing agents such as potassium bromate. This gel-forming characteristic of pentosans in response to bromate may promote normal dough rigidity and therefore enhance the cellular structure of bread. The unusual response of irradiated flour to bromate, whereby crumb grain remains poor while loaf volume is increased, may involve the damaging effect of radiation on pentosans.

In case of bread baked from formula 2 (rich formula without malt), the added sugar was required as food for the yeast. In formula 3 (rich formula without sugar), the added malt amylase hydrolyzed sufficient starch to supply the necessary fermentable sugar. Thus, the approximately equal loaf volumes representing different radiation dosages would be anticipated in formulas 2 and 3 with optimum bromate. In

producing bread from formula 4 (rich formula without sugar and malt), the fermentable substrates consisted of sugar in the flour and the relatively small amount of amylase activity in the flour. The greater volume of bread from 0.6×10^6 rep treatment, compared with the control, indicates that the radiation produced more fermentable sugar.

It is obvious that, although the starch fraction is seriously altered by radiation to the extent that fermentable sugars are produced, this degradation is not sufficient even at one million rep to eliminate the requirement in baking for additional sugar (for optimum fermentation and loaf volume), which is normally supplied either by added sugar or by an amylase enzyme supplement. This point has apparently been overlooked by European investigators (13), who have also observed the moderate loaf volume increase obtained when flour is gamma-irradiated, apparently using simple baking formulas in which sugar and malt are absent. Similar improving effects were observed previously by Milner and Yen (16) and more recently by Nicholas *et al.* (17). In the present studies, no indication was obtained that irradiation at any level improves bread properties when an optimum baking formula is used. Observations concerning apparent improvement (13,16,17) may, therefore, be considered to be valid only with reference to loaf volume when suboptimum levels of sugar, malt, or bromate are used in baking.

The starch degradation due to irradiation resulted in no increase in softness of bread crumb or in retardation of firming, which are benefits usually associated with moderate starch hydrolysis due to alpha-amylase supplementation in baking. In fact, and unexpectedly, radiation damage to starch significantly reduced crumb softness, and staling rate was increased in proportion to radiation dosage. These apparently anomalous results suggest that starch modification by irradiation is not a simple hydrolytic process but may also involve other complex chemical transformations.

The linear decrease in taste desirability of bread with the increase in radiation dosage, as revealed by the panel test, indicates that irradiation causes development of compounds that produce off-flavors in bread.

Additional investigation of possible effects of radiation on sulfhydryl groups in flour in relation to oxidation-reduction changes in dough seems warranted. Precise effects of radiation treatment of wheat and flour components probably can be approached best by investigating flour fractions individually irradiated.

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STUDIES WITH RADIOACTIVE TRACERS
III. The Effects of Defatting and of Benzoyl Peroxide on the Decomposition of Br^{82}-Labeled Bromate by Water-Flour Doughs[1]

C. C. Lee and R. Tkachuk

ABSTRACT

Decomposition of Br^{82}-labeled bromate to bromide by water-flour doughs prepared from commercially milled patent, intermediate, and clear flours that initially contained 15 p.p.m. labeled bromate showed that the lower the flour grade, the greater the decomposition of bromate to bromide. Defatting as well as treatment with benzoyl peroxide markedly decreased the amounts of bromate reduced to bromide for all the flours studied. Defatting also impaired the breadmaking properties of these flours, though both original and defatted flours showed responses to treatment with various levels of ordinary bromate. These results indicated that bromate, a nonspecific oxidizing agent, may take part in a number of oxidation-reduction reactions with oxidizable constituents of dough. These reactions may be involved in varying degrees of importance in the over-all effects of bromate on the final loaf characteristics of bread. If there were a particular bromate reaction with the defatted dough that might be termed as primarily responsible for the improver action, the present results would also indicate that such a reaction may consume only a small fraction of the bromate initially added to the flour.

There is considerable current interest in the role of lipids in flour quality. The literature on this subject has been reviewed in 1956 by Cookson and Coppock (2), and from their extensive experimental data these authors concluded that flour lipids are involved in both oxidative and crumb-softening types of bread improvement. More recently, Smith and co-workers (12,13) reported an interesting study on oxygen absorption and the effects of oxygen and fat on some physical and chemical properties of flour doughs. It was shown that polyunsaturated free fatty acids in the flour fat were intimately concerned with the changes observed in the effects of sulfur dioxide and bisulfite, extensogram patterns, and oxidation of sulfhydryl groups during dough mixing.

In the present work, the effects of defatting on the decomposition of Br^{82}-labeled bromate in water-flour doughs have been investigated. As an added variable, the influence of bleaching with benzoyl peroxide on the original and defatted flours was also studied.

Materials and Methods

Three grades of untreated flour commercially milled from Western Canadian hard red spring wheat were used in the experiments. These

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flours were designated patent, intermediate, and clear, which contained, respectively, 50, 38, and 12% of the total flour from the mill. Their protein and ash contents, on a 14% moisture basis, were as follows:

Grade of Flour	*Protein (N × 5.7)*	*Ash*
	%	%
Patent	12.4	0.35
Intermediate	14.0	0.46
Clear	16.7	0.76

Defatting of the flours was effected by extraction with petroleum ether, b.p. 40°–60°C., for 17 hours in a series of Goldfisch fat-extraction apparatuses. Batches of an extracted flour were thoroughly mixed and sieved to give a single stock supply to ensure uniformity when used in various experiments. The amounts of fat extracted were 0.69, 0.90, and 1.80%, respectively, for the patent, intermediate, and clear flours. The residual fats in the extracted flours were not determined.

For the benzoyl peroxide treatment, a commercial bleaching powder, Novadelox Type III, was used. This preparation contained 32% benzoyl peroxide with inert carriers consisting of potassium aluminum sulfate, calcium carbonate, dicalcium phosphate, and tricalcium phosphate. The original or defatted flour and 150 p.p.m. of this bleaching powder were mixed thoroughly in a MacLellan batch-mixer. Four days were allowed to pass after this treatment before the flours were used for studies on bromate decomposition.

Br^{82}-labeled potassium bromate was prepared as previously described (8). The water-flour dough was made from 100 g. of flour (at 14% moisture) and 60 ml. of distilled water containing 1.5 mg. (15 p.p.m.) of labeled potassium bromate. Duplicates of such doughs were prepared from each grade of the original flours, the defatted flours, the original flours treated with benzoyl peroxide, and the defatted flours treated with benzoyl peroxide. The doughs were kept at 30°C. for 4 hours and 7 minutes[2] after mixing, before they were extracted with water. Details of the extraction procedure have been given earlier (8). Bromide and bromate contents in these extracts were determined. The analysis consisted of measurements of the total Br^{82} activity in the extract, the activity remaining after the removal of bromide by exchange with bromine, and the activity remaining after the removal of both bromide and bromate, the latter by precipitation as silver

[2] The original intention was to have the doughs worked up 4 hours after mixing. However, in some early experiments, 7 extra minutes were required for certain manipulations and, consequently, all doughs were analyzed at 4 hours and 7 minutes after mixing.

bromate. The complete procedure and sample calculations have been outlined in paper II of this series (7).

Baking tests were carried out with the original and defatted flours. The formula used consisted of 100 g. flour (at 14% moisture), 3 g. yeast, 5 g. sucrose, 1.75 g. sodium chloride, 3 g. shortening, 4 g. nonfat dry milk, 0.1 g. ammonium dihydrogen phosphate, 0.3 g. nondiastatic malt, various levels of nonradioactive potassium bromate, and absorption to suit.

Results and Discussion

Data derived from radioactivity measurements are summarized in Table I. First of all, the total recoveries of Br^{82} in the aqueous extracts of all doughs studied are given in column 3. These recoveries are expressed as percentages of the initial bromate concentration of 15 p.p.m. Of the recovered Br^{82} in the aqueous extracts, the activities remaining after the removal of bromide and after the removal of both bromide and bromate are given in columns 4 and 5, respectively. These activities are expressed on a percentage basis by setting the recovered Br^{82} activity in a given extract as equaling 100%. The percent bromide in the extract of a given dough would thus be the difference between 100 and the corresponding figure in column 4, while the percent bromate would equal the difference between the figures in columns 4 and 5.

Recoveries of Br^{82} in Dough Extracts. The total recoveries of Br^{82} in the aqueous extracts of various doughs ranged between 84 and 100%. Most of these recoveries were over 90%, and, in some cases, complete recovery of 100% was noted. These results are in substantial agreement with the recovery of Br^{82} recorded in an earlier study (8). Very probably it is possible to extract all the Br^{82} activity from these nonfermenting doughs. The varying amounts of activity not recovered in the aqueous extracts may be due to losses arising from the small quantities of dough which adhere to the wall of the Waring Blendor and which are out of contact with the water during extraction.

Reduction of Bromate to Bromide by Doughs Made from Untreated Flours. For the original, untreated flours, marked differences in the amounts of bromate converted to bromide were found for the three grades of flour; the lower the grade, the greater the quantity of bromide in the aqueous extract. Evidently, more reducing materials capable of reacting with bromate are present in the lower-grade flours. Similar findings have been reported earlier (3,6,11). Furthermore, the greater ability of lower-grade flours in reducing bromate to bromide parallels the greater oxygen-uptake capacity of such flours (12). The

actual amount of bromide present in the aqueous extract of the patent flour dough was a little over 20% of the total activity in the extract. This is considerably higher than the bromide content of about 6%, 4 hours after mixing, in the aqueous extract of a nonfermenting water-flour dough made from a high-protein straight flour which contained initially 15 p.p.m. bromate (8). Such large variations further emphasize that different flours may have vastly different capabilities of reducing bromate.

Comparison of Bromide Contents in Defatted or Treated Flours

TABLE I

DECOMPOSITION OF Br^{82}-LABELED BROMATE TO BROMIDE BY WATER-FLOUR DOUGHS

Type of Flour	Grade of Flour	Recovery of Br^{82} in Aqueous Extract[a]	Br^{82} Activity in Aqueous Extract; Recovered Activity = 100%			
			After Removal of Bromide	After Removal of Bromide and Bromate	Bromide Content	Bromate Content
		% *I.B.*[b]	%	%	%	%
Original flour	Patent	96	79	7	21	72
		92	78	..	22	..
	Intermediate	91	69	..	31	..
		94	69	..	31	..
	Clear	100	33	..	67	..
		99	37	..	63	..
Defatted flour	Patent	95	90	7	10	83
		100	93	9	7	84
	Intermediate	97	88	7	12	81
		100	87	4	13	83
	Clear	96	77	6	23	71
		94	78	5	22	73
Original flour treated with benzoyl peroxide	Patent	100	79	6	21	73
		94	84	7	16	77
	Intermediate	87	81	8	19	73
		86	79	5	21	74
	Clear	87	61	8	39	53
		84	57	8	43	49
Defatted flour treated with benzoyl peroxide	Patent	98	95	3	5	92
		100	94	6	6	88
	Intermediate	95	90	4	10	86
		98	94	4	6	90
	Clear	90	74	4	26	70
		99	72	4	28	68

[a] All doughs were extracted at 4 hours and 7 minutes after mixing.
[b] Percentage of initial bromate (I.B.) concentration of 15 p.p.m.
[c] No measurements were made.

and in Untreated Flours. A comparison of the bromide content in aqueous extracts of doughs from the defatted or benzoyl peroxide-treated flour and from the original flour can be made quantitatively by the calculation of a bromide ratio. This may be defined as the ratio obtained by dividing the mean bromide content in the extract of dough from the defatted or benzoyl peroxide-treated flour by the mean bromide content in the extract of the corresponding dough from the original flour. Such bromide ratios (Table II) clearly indicate that defatting greatly decreased the amount of bromate reduced to bromide for all three grades of flour studied. This is in agreement with the

TABLE II

COMPARISON OF BROMIDE CONTENTS IN EXTRACTS OF DOUGHS FROM TREATED AND ORIGINAL FLOURS

TREATMENT	GRADE OF FLOUR	BROMIDE RATIO: TREATED FLOUR/ORIGINAL FLOUR[a]
Defatting	Patent	0.40
	Intermediate	.40
	Clear	.35
Benzoyl peroxide bleaching	Patent	.86
	Intermediate	.65
	Clear	.63
Defatting and benzoyl peroxide bleaching	Patent	.25
	Intermediate	.26
	Clear	0.42

[a] Mean bromide content in extracts of doughs from treated flour/mean bromide content in extracts of doughs from original flour.

recent findings of Cunningham and Hlynka (4) that the rate of bromate loss is depressed by defatting. Similar decreases in the reduction of bromate to bromide, though to a lesser extent, resulted from treatment of the flours with benzoyl peroxide. With flours subjected to both defatting and peroxide treatment, the lowest conversion of bromate to bromide was found for the patent and intermediate flours. However, the data appear to indicate that the clear flour, subjected to both defatting and peroxide treatment, resulted in the conversion of bromate to bromide in about the same order of magnitude as by defatting alone.

It is quite evident from the above considerations that a large fraction of materials capable of reducing bromate to bromide, directly or indirectly, could be extracted from the flour by petroleum ether. This observation again parallels the finding of Smith and Andrews (12) that removal of lipids by solvent extraction decreased oxygen uptake during mixing of water-flour doughs. While Smith and Andrews have demonstrated that polyunsaturated free fatty acids in the flour extract may be

responsible for the uptake of oxygen, the present authors have not yet investigated the nature of the compound or compounds extracted from flour to which the effects on the decomposition of bromate may be attributed. In 1940, Balls and Hale (1) and Sullivan (14) reported the occurrence of a sulfur-containing constituent, probably bearing a reversibly oxidizable sulfhydryl group, in the petroleum ether extract of wheat flour. Removal of such a constituent may partially account for the observed effect that defatting markedly decreased the amount of bromate reduced.

Benzoyl peroxide is generally believed to exert its bleaching effect through its action on the carotenes (5). Fairly recently, it was also found that benzoyl peroxide can affect the "residual pigment," which showed primary absorption in the UV region (10). Since bromate is not a bleaching agent, presumably bromate will not act on the flour pigments as does benzoyl peroxide. The radioactivity data, indicating that benzoyl peroxide treatment of the flours gave rise to decreases in the amounts of bromate reduced to bromide, strongly pointed to the presence of some reducing substances in water-flour doughs which could react with either bromate or benzoyl peroxide. The pronounced effects of defatting as well as of benzoyl peroxide treatment on the capacity of the dough to convert bromate to bromide may, therefore, be regarded as indicating that bromate, a nonspecific oxidizing agent, may take part in a number of oxidation-reduction reactions with various oxidizable constituents of dough.

Bromate Contents in Extracts of Various Doughs. The discussion so far has dealt only with the amounts of bromate reduced to bromide. The nonbromide fraction of the Br^{82} activity in the aqueous extracts of various doughs may be presumed to be mostly unchanged bromate. This was actually found to be so (Table I). Removal of both bromide and bromate from aqueous extracts of doughs was carried out for all doughs from defatted and benzoyl peroxide-treated flours and for one dough sample from the original patent flour. The residual activities after the separation of both bromide and bromate amounted to only 3–9% of the recovered activities in various dough extracts. This residual activity may possibly be partly due to incomplete precipitation of the silver bromate; however, further investigation is needed to elucidate its exact nature.

Relationship between Flour Lipids and the Improver Action of Bromate. The nature of the relationship between flour lipids and flour quality is still uncertain. In their literature review, Cookson and Coppock (2) quoted seven references reporting improvement and three references reporting impairment in the breadmaking properties

of ether-extracted flour. For the flours used in the present investigation and under the experimental conditions of defatting and test baking described earlier in this paper, impairment in both loaf volume and crumb texture resulted from defatting the flours. With added bromate, improvements were noted for bread made from the original as well as the defatted flours. Data from a typical bake are given in Table III. These results are in agreement with the findings of Sullivan and coworkers (14,16).

The ability of defatted flours to respond to bromate improvement may lead one to believe, as did Sullivan (14), that lipids, or rather the ether- or petroleum ether-extractable constituents of flour, are not primarily responsible for the beneficial effects of bromate. Since bromate may undergo a number of oxidation-reduction reactions in the dough system, some of these reactions may be more concerned than

TABLE III

BAKING TESTS WITH ORIGINAL AND DEFATTED FLOURS

FLOUR	BROMATE LEVEL	LOAF VOLUME	CRUMB TEXTURE	CRUMB COLOR
	ppm	*cc*	*score*[a]	*score*[a]
Original, patent	0	800	8	8
	5	825	8	8
	10	835	8	8
	15	850	8	9
	20	845	7	9
Defatted, patent	0	755	6	10
	5	775	6	10
	10	795	9	10
	15	765	8	10
	20	780	7	10
Original, intermediate	0	835	8	8
	5	880	8	8
	10	860	8	8
	15	935	8	8
	20	960	9	9
Defatted, intermediate	0	750	6	9
	5	760	6	9
	10	810	6	9
	15	805	6	9
	20	840	7	10
Original, clear	0	740	5	3
	5	815	5	3
	10	865	6	4
	15	910	8	4
	20	940	8	5
Defatted, clear	0	670	5	4
	5	750	5	4
	10	740	5	5
	15	740	5	6
	20	850	5	6

[a] Scoring of crumb characteristics referred to a high quality standard assigned a score of 10.

others with the over-all effects of flour improvement. If there were a particular reaction that might be termed primarily responsible for the improver effect, such as the possibility of formations of cross-linkages involving the sulfhydryl and disulfide groups (15), this reaction might consume only a small fraction of the bromate initially added to the flour. Other reactions in which bromate may take part, such as those indicated by the results presented in this paper – namely, the reduction of bromate by solvent-extractable constituents of flour and reactions of bromate that may be influenced by benzoyl peroxide treatment – may all be involved in varying degrees of importance in the over-all effects of bromate on the final loaf characteristics of the bread.

The over-all effects of bromate on the loaf characteristics of the finished bread probably are dependent on many complex factors, including factors loosely termed by Martin and Whitcomb (9) as the chemical constitution and the physical-chemical environment of the gluten. Although both our original and defatted flours responded to bromate, for a given bromate treatment, the loaf volumes of bread from the original flours were always higher than those from the defatted flours (Table III). In conjunction, it was found from the tracer studies that defatting definitely removed materials which could react, directly or indirectly, with bromate. These facts strongly indicated that reactions of bromate with petroleum ether-extractable constituents of flour, though perhaps not primarily responsible for the improver effect, are involved, probably by influencing the physical-chemical environment of the dough protein network, in the over-all effects of bromate on the loaf characteristics of bread.

Acknowledgment

The technical assistance of R. G. Teed is gratefully acknowledged.

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CONSISTENCY MEASUREMENTS ON BATTERS, DOUGHS, AND PASTES[1]

E. B. Lancaster and R. A. Anderson

ABSTRACT

A consistency-recording apparatus, consisting of a variable voltage transformer and a recording differential ammeter, has been used with various mixers to investigate some mixing properties of a variety of batters, doughs, and pastes over an extremely wide consistency range. Curves obtained when flour-water batters were tested have proved to be valuable in predicting the proper water-flour ratio needed for carrying out the continuous batter process for fractionating wheat flour. Also from these curves, it has been possible to establish a relationship between the protein content of the flour and the consistency of the batter. The relationship, based on 41 observations, has a correlation coefficient of 0.97, and a standard error of estimate of 0.104, in terms of consistency. To demonstrate the operating range of the apparatus, it has been applied to the examination and testing of cake-mix batters, starch-water mixtures, and doughs prepared from dried gluten and soft wheat flour.

More comprehensive results have now been obtained with a consistency-recording apparatus for measuring mixing characteristics of flour-water batters which has been described by Anderson and Lancaster (2).

[1] Manuscript received September 8, 1958. Contribution from the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

This apparatus was designed and fabricated to obtain information which would be useful in operating the batter process—a process for separating gluten and starch in wheat flour by diluting a mixed batter with water and screening the resulting slurry.

This paper describes further application of the apparatus to flour-water batters and illustrates the flexibility of this device when applied to other mixtures containing starch and water. It also discusses the value of information on mixing characteristics of batters in successful operation of the batter process.

Materials and Methods

The apparatus used for testing flour-water batters consists of a variable voltage transformer, a differential recording ammeter, and a Model C Hobart[2] mixer equipped with a "B" beater. A number of flours representing patents from four types of wheat, second clears, and synthetic compositions of a single flour with varying amounts of wheat starch were examined. A total batter weight of 3,500 g. was used, and the temperature of the water was 53°C. A drop of about 10°C. was encountered in the first ten minutes of mixing, because of cooling. The amounts of water and flour used to prepare the batters were calculated from the formulas:

$$F = 3{,}500/(1 + R)(1 - p)$$

$$W + F = 3{,}500$$

where F is the g. of flour, as is;
R is the desired water to flour ratio, parts of water/part of dry flour;
p is the moisture content of the flour, expressed as a decimal; and
W is cc. of water added.

The same apparatus and formulas were used in measuring the mixing properties of the flour-water doughs and mixtures, as well as mixtures prepared with ungelatinized wheat starch.

Cake-mix batters were tested by connecting the recording device to a Hobart Kitchen-Aid mixer, Model K4-B, which was driven by a 1/6-h.p., 115-v., AC-DC universal motor. The mixer was operated at 70 volts, with a speed setting of 4. About 530 g. of mix were used. The tests with gelatinized starch required the use of a sigma-blade mixer operating at 39 r.p.m. and powered through a gear reducer by a 1/4-h.p., 115-v., 60-cycle, AC induction motor. Batch size was held at 500 g. Water at approximately 25°C. was used in both cases.

[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

Glutens dried by different heat treatments were tested in a 50-g. Brabender Farinograph mixer bowl, driven at 59 r.p.m. through a gear reducer by a 1/6-h.p., 115-v., 60-cycle, AC induction motor. The recording device was connected to this motor. For the test, 20 g. of gluten and 15 g. of soft wheat flour were placed in the mixer, blended dry, and the instrument was zeroed. Twenty-five milliliters of water were added, and the mixing characteristics of the mixture were recorded for 8 to 10 minutes. Erratic results were obtained when starch was used in place of soft wheat flour, because the starch-gluten mixture refused to stay in the bowl during the first stages of mixing.

The motors on the various mixers were allowed to operate sufficiently long, from 30 to 60 minutes, for the no-load current to become constant before making tests.

The moisture of the flour was determined by drying a sample for 4 hours at 110°C. under a vacuum of 28 inches of mercury. Protein (N × 5.7) was determined by the Kjeldahl-Gunning-Arnold method (1).

Definitions

For convenience in discussing the curves obtained with the consistency-recording apparatus, certain properties and measurements have

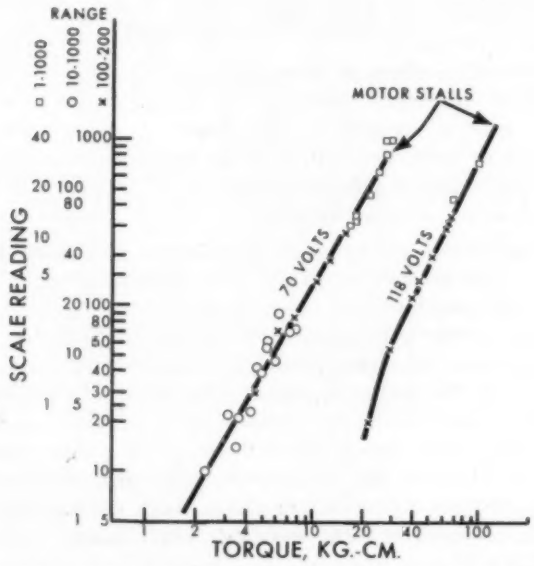

Fig. 1. Calibration curves for consistency recorder at 70 and 118 volts (Hobart mixer).

been arbitrarily defined. The definitions apply only to this paper.

The property which is measured by the apparatus will hereafter be termed *consistency*, whether the viscosity of thin pastes or the plastic behavior of doughs is being described. The degree of consistency, termed the *consistency value*, is expressed as the scale reading of the recording instrument when it has been converted to the arbitrary scale represented by operation of the transducer with the 100-ohm shunt, the Model C Hobart mixer at 70 volts, and the meter sensitivity setting at 200 microamperes. The scale readings at other range settings are shown in Fig. 1. Scale readings in the 100–200 range (100-ohm shunt, 200 microamperes meter sensitivity), using the 70-volt line or its extension, are the consistency values. The other ranges refer to other combinations of transducer and meter sensitivity settings.

The term *consistency index* is restricted to mean the consistency value observed on a flour-water batter or dough after it has been mixed for 5 minutes. At this point the consistency value is near a minimum point in the mixing curve.

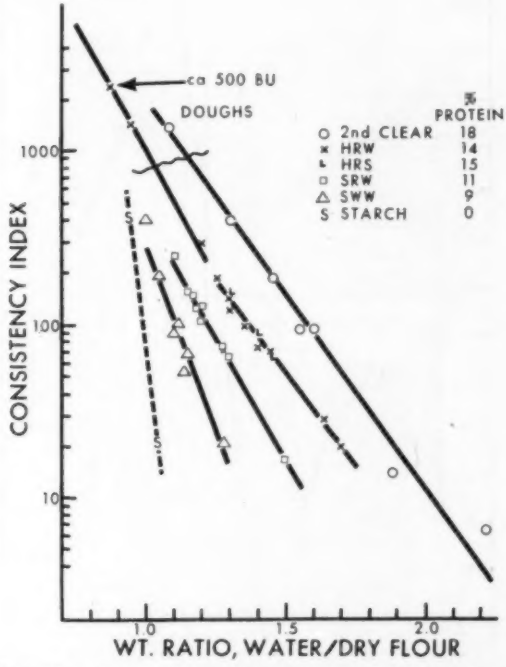

Fig. 2. Effect of water on the consistency index of various flours and starch (Hobart mixer).

Results on Flour-Water Batters

Considering only a single flour, the consistency indices are closely related to the amount of water in the batter, as seen in Fig. 2. Further, if flour-water mixtures in the ordinary batter range are examined, the effect of protein content also becomes evident. Data in Fig. 2 and additional data from synthetic flours were employed in a multiple regression calculation leading to the expression:

$$\log C = 6.099 - 0.1053\,(W - 3.15P)$$

where C = consistency index;

$W = (R \times 100)/(1 + R)$ = percent water in the batter;

P = percent protein in flour (moisture-free basis)/ $(1+R)$ = percent protein in batter; and

R = water/flour ratio, parts of water/parts of dry flour.

This relationship, based on 41 observations, has a correlation coefficient of 0.97 and a standard error of estimate in terms of log C of 0.104. Although the precision for estimating protein from consistency

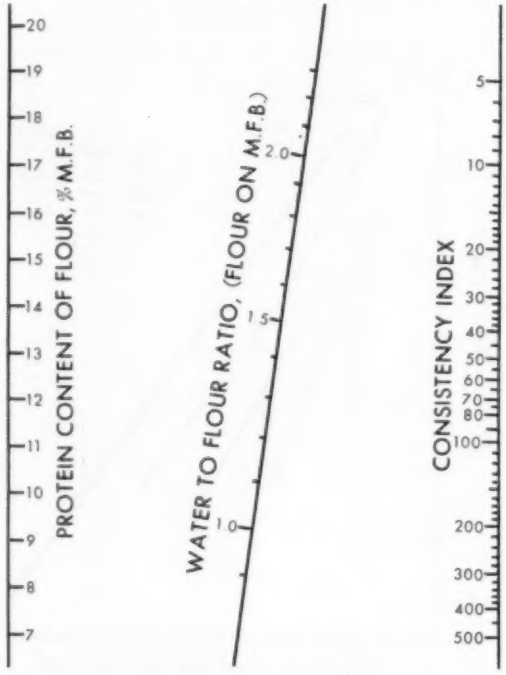

Fig. 3. A nomographic solution for the general consistency index equation (Hobart mixer).

indices and water content is somewhat different, it still compares favorably with that as reported from sedimentation or biuret methods, e.g., by Miller *et al.* (4) and Pinckney (5). A nomograph that may be used to solve the equation is given in Fig. 3. Water content of the batter is expressed as the weight of water per unit weight of dry flour. It must be remembered that the equation was obtained with a relatively small number of flours, but it illustrates the general relationships involved. Also, the values were obtained with our particular combination of differential recording ammeter and Model C Hobart mixer equipped with a "B" beater.

Besides furnishing an estimate of the quantity of protein in a flour, qualitative differences are very apparent in mixing curves obtained with the apparatus, as demonstrated in Fig. 4. The flours tested were all of second clears grade and all had about the same protein content. The sensitivity setting was such that the maximum scale reading represents a consistency value of 500. Consistency indices are obviously influenced somewhat by the rate of development caused by qualitative

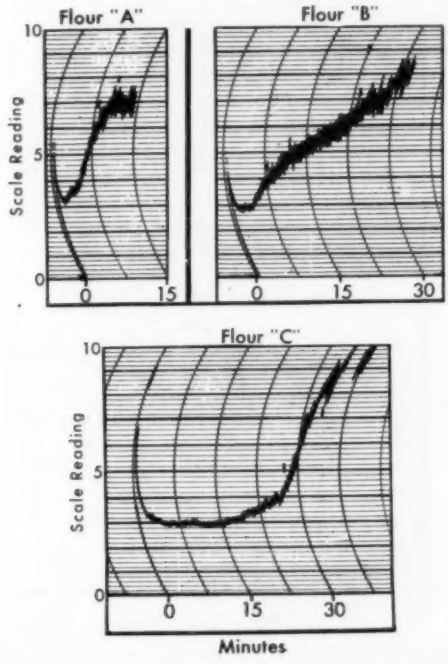

Fig. 4. Qualitative differences between flours of the same grade and protein content.

factors, and the minimum value may be a better indication of the protein content than the consistency index. The amount of water used in these tests, 1.5 parts per part of dry flour, yielded fairly stiff batters, and the resultant curves are typical of such batters.

Results on Other Mixtures

The consistency values and their change with mixing time are compared in Fig. 5 for two batters, a starch-water mixture, and a dough. These curves were redrawn on a semilog scale from curves similar to those in Fig. 4, and were from data obtained with the Model C Hobart mixer. The curves indicate the qualitative differences that may be expected between various mixtures, and they emphasize the differences in information about flours that may be derived from examination of batters, as opposed to the examination of doughs by the farinograph or mixograph. Little attention is paid to the minimum often obtained in dough-mixing curves with a farinograph. In contrast, the minimum point is very important in batter-mixing curves; since it indicates the length of time that the batter must mix before develop-

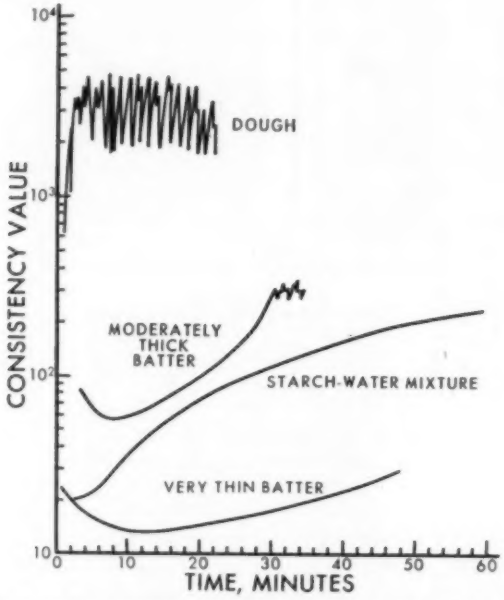

Fig. 5. Relationship between consistency value and mixing time of a dough, batters, and a starch-water mixture.

ing or thickening, the point becomes an important factor in evaluating a flour for use in the batter process. That portion of the batter-mixing curve after the minimum is also important because it shows the speed of thickening.

With mixers other than the Hobart, the range of consistency values may be extended above and below the region covered in Fig. 5. For mixtures of the consistency of doughs, or for more plastic mixtures, the low-speed, sigma-blade mixer performs satisfactorily, although there are obvious differences in mixing action between this and the Hobart mixer. Typical curves for the mixing characteristics of "pastes" made from gelatinized starch containing 32 and 42% solids are shown in Fig. 6. The maximum consistency value for the 42% mixture is several times that of an ordinary bread dough.

For mixtures of lower plasticity, sensitivity may be achieved by using a mixer with a less powerful motor. However, measurements in this region are already covered fairly well by viscometers, operating on similar principles, and commonly employing a simple agitator-type

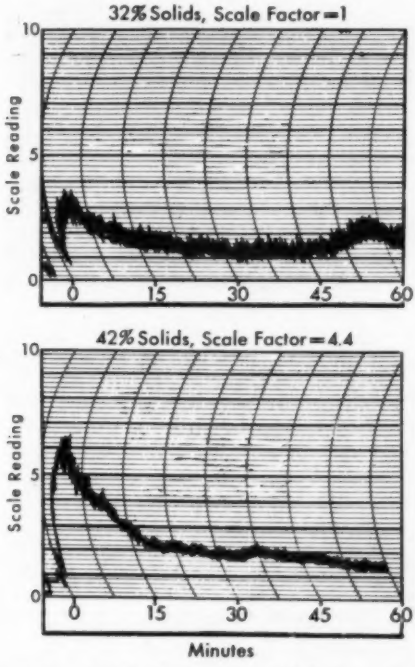

Fig. 6. Properties of stiff mixtures of gelatinized starch and water.

mixer. Also, reduction in batch size is often desirable. The best results are obtained by reducing the motor size as well as the size of the bowl and mixer blade. Often these changes introduce the question of the nature of the mixing action. For example, in an investigation of the properties of cake-mix batters, both a Hamilton-Beach and the Kitchen-Aid mixer were employed. While the shape of the curve for the cake batters was independent of the mixers, the conventional kitchen beater such as that on the Hamilton-Beach machine was entirely unsatisfactory for flour-water batters because of the tendency of the batter to wind around the beater shafts. Examples of results obtained in the first-stage mixing of three-stage cake batters using the Kitchen-Aid mixer are presented in Fig. 7. The consistency of these mixtures is in about the same range as that of a very thin flour-water batter, although the water-solids ratio is much lower.

Typical mixing curves obtained from mixtures of dried wheat gluten, soft wheat flour, and water are shown in Fig. 8. One curve was

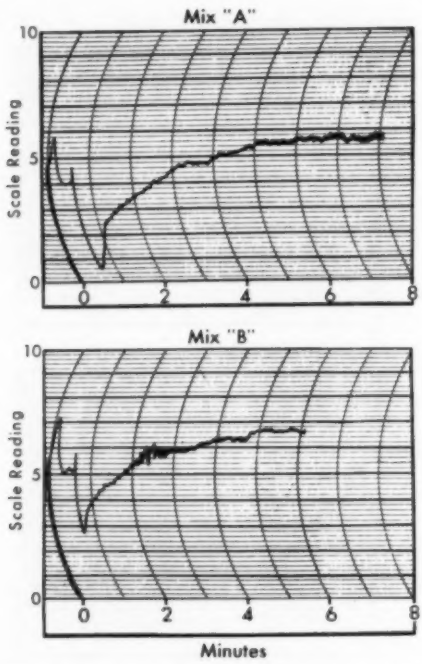

Fig. 7. Development curves in the first-stage mixing of two three-stage cake-mix batters.

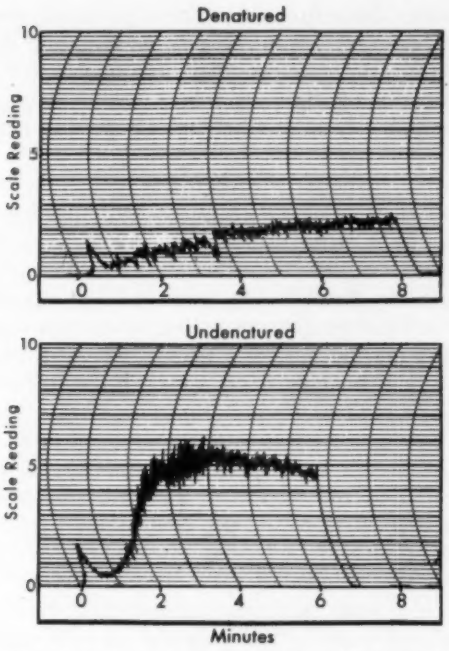

Fig. 8. Properties of synthetic doughs consisting of denatured or undenatured wheat gluten, a soft wheat flour, and water.

obtained using substantially undenatured gluten that was prepared by drying wet gluten under a vacuum of 29½ inches of mercury at 35°C. for 16 hours. The second curve was obtained using gluten that was dried from the same batch of wet gluten, but at a considerably higher temperature so that it was considerably denatured. Hydration of the mixture containing the undenatured product proceeded rapidly to a maximum consistency value well over twice that of the mixture containing the denatured material. Hydration of the denatured gluten proceeded very slowly.

Discussion

Consistency curves permit a prediction of the power requirements necessary to prepare a given mixture, and they also permit an estimation of the mixing energy for a given process. In addition, the curves furnish information about differences in raw materials so that the process may be adjusted to meet demands of differing input material, or so that the input material may be controlled by specification or

blending to meet the requirements of a standard process. Because not enough experience has been gained in applying the consistency-recording apparatus to mixtures other than flour-water batters to permit an interpretation of all results, this discussion is limited to the way in which information from mixing curves is used to help achieve success in operating the batter process.

In the continuous batter process it has been found that, although farinograph information on a flour is a rough indication of the quality of the flour and thus of the degree of success to be expected by operating with a standard procedure, batter curves are more informative for control purposes. Batch studies indicated that mixing a batter sufficiently long so that the consistency value before dilution rises to about twice the consistency index is more than sufficient for good recovery of gluten, regardless of the consistency index of the batter. However, experience in the pilot plant (3) indicates that the time to the minimum point on the mixing curve is a better criterion of probable success. In a continuous plant this time should be somewhat less than the effective retention time of fresh feed in the particular mixer used. Excellent gluten recovery is obtained in the continuous batter process pilot plant, when the time to the minimum obtained in the mixing curve is about 2.5 minutes. The time to the minimum may be shortened most effectively by increasing the batter temperature, but decreasing the amount of mixing water is also effective, as seen in Fig. 5, and is usually the control used in the operation of the continuous pilot plant.

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OBSERVATIONS ON THE POTENTIOMETRIC TITRATION OF SULFHYDRYL GROUPS IN WHEAT GLUTEN WITH IODINE[1]

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ABSTRACT

An attempt was made to develop a method for the direct titration of gluten sulfhydryl groups with iodine in dilute acetic acid. The procedure which evolved offers a suitable method for making comparative studies of flour constituents; however, some question remains as to whether it provides an absolute measure of sulfhydryl groups. Values obtained for sulfhydryl-containing proteins were consistently higher than those by other methods, although correct values were obtained for glutathione, 2-mercaptoethanol, and the sulfhydryl-free protein, casein.

The problem of gluten solubility was overcome by the use of a titration medium having low ionic strength, and interference of turbidity with detection of end point was avoided by the use of a potentiometric method. The standard deviation of results based on differences between duplicate titrations was 5.4% of the average titer.

Glutens from a variety of sources were found to reduce from 6.4 to 9.8 μeq. of iodine per g. of protein. Variations were observed among the constituents of flours that had different baking qualities.

Widespread interest for many years in oxidation-reduction processes of flour and dough caused the investigation of sulfhydryl groups of gluten by a number of procedures (2,14,23,28,31,37). One of the chief obstacles encountered has been the insolubility of gluten in the presence of salts. Matsumoto and Shimoda (31) titrated intact gluten in a medium containing potassium chloride by using urea to prevent precipitation. Kong, Mecham, and Pence (28) solubilized gluten by digestion with trypsin. The present procedure avoids the solubility problem by providing a salt-free titration medium.

Another difficulty encountered in working with gluten is the turbidity of its dispersions, which interferes with colorimetric procedures and with titrimetric procedures involving a color change at the end point. This difficulty was avoided in the present method by determining the end point potentiometrically.

Iodimetric methods have been criticized because of 1) possible overoxidation of sulfhydryl groups beyond the disulfide stage and 2) possible reaction with other functional groups. Therefore, the reaction of iodine was studied, not only with sulfhydryl compounds but also with other substances that might be suspected of reacting with iodine; values are given for the apparent sulfhydryl content of glutens from a va-

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riety of sources; and finally, the distribution of iodine-reducing capacity among the constituents of flours of differing baking quality is reported.

Materials and Methods

A Cambridge electron ray pH meter[2] equipped with a calomel-platinum electrode pair was used for emf. measurements. The sample was contained in a 250-ml. beaker which was fitted with a rubber cover having holes for the admission of electrodes, stirrer, buret tip, and nitrogen inlet tube. All solvents were purged with nitrogen for 30 minutes at room temperature.

The iodine reagent was prepared by diluting a saturated solution of iodine in 0.01*N* acetic acid to the desired concentration and standardizing against sodium arsenite in the usual manner. Although deterioration of iodine solutions was considerably slowed by nitrogen purging, the titrant should be checked daily for greatest accuracy. The range of the method is flexible, being dependent on the iodine concentration. For example, with $3 \times 10^{-4}N$ iodine, 1 g. of gluten or 10 g. of flour can be titrated with 20 to 50 ml. of reagent.

Titrations were conducted under a stream of nitrogen with the sample dissolved in 0.01*N* acetic acid. It was convenient to add iodine reagent in increments amounting to approximately one-tenth of the total titer. Values of emf. measured to the nearest mv. 3 minutes after each addition of iodine were plotted against the volume of iodine added, and the end point was indicated by a well-defined inflection in the resulting curve. A negligible blank was encountered in most cases. Titrations should be carried out at room temperature, or below, and temperature variations should be minimized (26). Changes in pH between 3 and 6 had no detectable effect unless accompanied by changes in ionic strength. Ionic strength should be kept low, as will be discussed later. A dilution effect was noted in results with reduced glutathione (increased titer at concentrations lower than 200μM) as well as with cysteine, but not with proteins. Antifoam agents had to be used with caution. For example, octyl alcohol did not interfere with titrations when present at a concentration of 200 to 350 p.p.m., but Dow-Corning AF antifoam, which was studied in the range of 20 to 12,000 p.p.m., gave erratic results.

Flours were selected as representative of good-baking hard red winter wheat (Ponca), poor-baking hard red winter wheat (Red Chief), good-baking hard red spring wheat (Lee), and poor-quality durum

[2] Mention of firm names or trade products does not constitute endorsement or rejection by the U. S. Department of Agriculture over others of a similar nature not mentioned.

(Golden Ball). They were ground from pure variety lots of wheat in an experimental Buhler mill under nearly identical conditions to the following extraction rates: Ponca, 69%; Red Chief, 63%; Lee, 71%; and Golden Ball, 55%. Following extraction with anhydrous *n*-butyl alcohol, they were prepared for titration by agitation in 0.01*N* acetic acid for 3 minutes in a Waring Blendor under a stream of nitrogen. Nongluten constituents of flours were isolated by centrifuging gluten wash liquors, thus separating starch from solubles.

Glutens were obtained from a variety of sources. Fresh glutens were hand-washed from flours with 0.1% aqueous sodium chloride by the method of Dill and Alsberg (15). Commercial glutens were received from Huron Milling Co. (Huron Gum Gluten, Lot 151) and Keever Starch Co. (Kesco Vital Wheat Gluten). Gluten samples were prepared for titration in the same manner as flour samples.

Crystalline egg albumin (No. 7300), bovine beta-lactoglobulin (No. 1616), 2X crystalline edestin (No. 8174), and Hammersten quality casein (No. 1479), were obtained from Nutritional Biochemicals Corporation. A sample of bovine serum albumin (Armour, No. P67908) was also used. All the proteins dissolved readily in 0.01*N* acetic acid, except egg albumin which was dissolved in distilled water prior to titration.

The following sulfhydryl compounds were used: Reduced glutathione, chromatographically pure, No. 10391, and L-cysteine (free base), chromatographically pure, No. 2534, from Mann Research Laboratories, Inc.; and 2-mercaptoethanol, No. 4196, from Eastman.

Results and Discussion

Influence of Electrolytes. Values obtained for reduced glutathione in the presence of 1*N* concentration of a number of selected electrolytes are presented in the following table:

Electrolyte	*pH*	*Iodine Reduced*
		eq/mole
Potassium bromide	3.3	2.16
Sodium chloride	3.2	1.92
Hydrochloric acid	0.15	1.92
Ammonium chloride	3.2	1.83
Sodium acetate	6.4	1.82
Ammonium acetate	6.5	1.80
Sodium iodide	4.0	1.06
Potassium iodide	3.3	1.06

Most of the salts caused an increase in iodine titer, and the extent of increase was dependent on the anion rather than the cation. Ionic effects predominated over pH effects as shown by the similarity of re-

sults obtained with chlorides and acetates despite the difference in pH.

Since the alkali metal iodides did not increase the titer of reduced glutathione, a study was made of the effect of potassium iodide on other sulfhydryl-containing substances. Urea was added to the solvent system at a concentration of 25 g. per 100 ml. (3.5*M*) of 0.01*N* acetic acid in order to prevent precipitation of gluten in the presence of the salt (31). The following values show the effect of adding urea alone and in combination with 0.5*N* potassium iodide:

Sample	*Urea alone*	*Urea plus potassium iodide*
	μeq/g	*μeq/g*
Kesco Vital Wheat Gluten	11.1	1.2
	μeq/mole	*μeq/mole*
Beta-lactoglobulin	7.1	3.0
2-Mercaptoethanol	1.10	0.70
Reduced glutathione	1.10	1.02

The effect of potassium iodide was variable, depending on the substrate. In view of the inconsistent effects of electrolytes and since similar unexplained behavior has been reported by Burk (11), it is concluded that most reliable results can be expected under conditions of low-ionic strength.

Specificity, Stoichiometry, and Reproducibility. Since it is known that iodine can react with a number of functional groups under the proper conditions of temperature, pH, and concentration, an investigation was made of the reaction of iodine with several selected substances under the mild conditions used in this method. The amino acids, histidine, tryptophan, and tyrosine, either alone or in combination with gluten or reduced glutathione, did not interfere with iodine titrations when present in quantities normally encountered in proteins. These results were substantiated by the fact that casein, which does not contain sulfhydryl groups but which contains the three amino acids, did not reduce iodine. Wheat lipids isolated from *n*-butyl alcohol extracts of flour did not reduce iodine.

Wheat starch reacted with 0.1 μeq. of iodine per g. of starch, an amount too small to interfere with titrations of crude gluten which contains approximately 10% starch. Flour solubles, however, reduce iodine even when free of protein (1,17). Thus, the present method cannot be employed in measuring sulfhydryl groups in whole flour samples. Results with glucose indicate that "reducing" sugars do not interfere. The only substances known to reduce iodine under the conditions used are reductones (39). Qualitative tests with acidic silver nitrate (25) did not indicate the presence of reductones in untreated

flour, though they are reported to exist in roasted flour (40) and could be expected to form in the presence of high concentrations of reducing sugars (30,32). The use of *N*-ethyl-maleimide and sodium iodoacetate to test the specificity of iodine titrations was abandoned when these reagent failed to react consistently with sulfhydryl-containing substances. They completely blocked the sulfhydryl groups of reduced glutathione and beta-lactoglobulin, but they reacted with only one group per mole of egg albumin, whereas several times this number are reported to be present (see Table I). Tests with *p*-chloromercuribenzoate were not possible because of side reactions with iodine.

Titrations of low-molecular sulfhydryl compounds yielded the following values:

Compound	*Iodine Reduced*
	eq/mole
Cysteine	1.8 to 3.0[a]
Reduced glutathione	1.19
2-Mercaptoethanol	1.06

[a] The titer for cysteine varied widely with concentration. The first value was obtained at a concentration of 7.9×10^{-4} *M*; the second, at 1.6×10^{-4} *M*.

These results agree with literature reports that cysteine is easily oxidized beyond the disulfide stage (8,29,35). However, with glutathione, in which the free carboxyl and amino groups are more remote, and with a simple compound like 2-mercaptoethanol, which contains no ionic groups, very close to one equivalent of iodine is reduced per sulfhydryl group (29). Furthermore, Berger, Noguchi, and Katchalski recently found that experimentally prepared poly-L-cysteine was not overoxidized by iodine (7). Therefore, it is reasonable to assume that normally one equivalent of iodine is reduced per protein sulfhydryl group.

The reproducibility of results from the present method was entirely satisfactory. The standard deviation based on differences between duplicate titrations was calculated according to the equation,

$$\text{standard deviation} = \sqrt{1/2n \text{ (the sum of } d^2)}$$

(41), where n is the number of sets of duplicate titrations and d is the difference between duplicates, which is taken for each set of the n pairs. For 41 pairs of duplicates having an average titer of 24.7 ml. the standard deviation was 1.34 ml.

Proteins. A comparison is made in Table I of results obtained by the present procedure with published data from other procedures. Values are consistently higher than those from other methods; in most cases these differences are too great to be explained by differences in

TABLE I

SULFHYDRYL CONTENTS OF PROTEINS (EQUIVALENTS PER MOLE) AS DETERMINED BY SEVERAL METHODS

PROTEIN	PRESENT PROCEDURE	OTHER PROCEDURES			
		Iodimetric	Porphyrindin	Amperometric, Ag-Tris	PCMB (9) [a]
Beta-lactoglobulin	4.0	3.8 (29)	1.6 (22)		2.2
Egg albumin	6.7	5.7 (29)	4.9 (19)	5.0 (6)	4.0
Bovine serum albumin	2.9	1.8 (24)	1.9 (21)	1.02 [b]	0.85
Edestin	3.3	2.0 (24)	1.9 (20)	.....	.....
Casein	0	0 (29)	0 (21)	.....	.....

[a] Numbers in parentheses indicate literature citations.

[b] Unpublished data, D. K. Mecham.

sample preparation. Since reasonable agreement was obtained in the case of low-molecular compounds, a possible explanation for the lack of agreement among protein values is that steric and inductive effects in the protein molecule either promote overoxidation of sulfhydryl groups by iodine, or inhibit reaction with other reagents (3,4,5,10,12, 16). The former possibility has been discussed earlier. An example of the latter possibility was reported by Fraser, Owen, and Shaw (16) in a study of the polarographic analysis of various sulfhydryl compounds. They concluded that " . . . in the absence of a carboxyl group the polarographic detection of thiols is not possible with the methods at present available." Furthermore, Kolthoff and co-workers recently reported, for the first time in the literature, evidence that low values can be obtained with amperometric titrations of proteins that have been denatured under conditions which allow cross-linking (27). Porphyrindin values are subject to the limitations of the nitroprusside reaction because the latter was used as external indicator. The lack of agreement of the present method with other iodimetric methods may be due to the fact that other authors conducted their titrations in the presence of electrolytes, with the possible complications already discussed (11).

Another possibility is that the present results may be high because of the presence of some unidentified reducing material associated with proteins, which does not interfere with other sulfhydryl methods. For example, reductones may be present in flour proteins as a result of sugar-protein interaction (30) during drying, milling, and storage operations. Such compounds, however, have not been demonstrated in untreated flour; and, if present, they would also interfere with existing amperometric methods, since the heavy-metal ions used in these methods are reduced by reductones.

Gluten. Titrations of glutens from a variety of sources gave:

Gluten	*Iodine Reduced*
	μeq/g
Laboratory-prepared, hand-washed:	
Ponca	6.4
Red Chief	6.8
Golden Ball	8.9
Lee	9.2
Commercially prepared:	
Huron Gum Gluten	9.8
Kesco Vital Wheat Gluten	8.6

Results for the laboratory glutens do not indicate any correlation between baking quality and amount of iodine reduced. Glutens from the winter wheats, however, reduced considerably less iodine than those from the spring wheats. Values for the commercial glutens are similar to those for the laboratory preparations despite the fact that they probably contain somewhat larger proportions of starch and lipid. This similarity is not surprising because other studies mentioned above did not indicate interference by these substances.

Literature values for the sulfhydryl content of gluten vary widely. Ferrometric methods give values from 0.4 to 31.1 μeq. sulfhydryl per g. protein (2,13,23). The highest values, 23 to 50 μeq. per g., were obtained polarographically (14); however, De Lange admits that the values may be high because of possible reduction of disulfide bonds to sulfhydryl groups in the ammoniacal-cobalt medium used. Matsumoto and Shimoda (31), with an amperometric method using urea to prevent precipitation of the gluten, found 1.6 to 2.0 μeq. per g. in a medium of dilute acetic acid.

The effect of urea on the apparent sulfhydryl content of gluten and other sulfhydryl-containing substances is shown in the following table:

Sample	*Acetic Acid*	*3.5M Urea*
	μeq/g	*μeq/g*
Kesco Vital Wheat Gluten	8.6	11.1
	eq/mole	*eq/mole*
Bovine serum albumin	2.9	3.8[a]
Beta-lactoglobulin	4.0	7.1
Reduced glutathione	1.19	1.10

[a] 6*M* urea.

Since the titer for reduced glutathione was not raised by adding urea, the increased values for gluten and the other proteins can be ascribed to greater availability of the sulfhydryl groups in the latter.

Disulfide bonds of gluten are easily cleaved by alkali, as would be expected (34,37,38). Treatment at pH 11 for 30 minutes in a medium containing 25% urea increased the apparent sulfhydryl content of a

gluten sample 42%. Under these same conditions, reduced glutathione lost 79% of its reducing power and 2-mercaptoethanol lost 46%.

Other Flour Constituents. As indicated above, there appeared to be no correlation between the reducing power of glutens and the baking properties of their respective flours; consequently, a study was made of the distribution of iodine-reducing capacity among the main constituents (gluten, starch, and solubles) of these flours. Table II gives the amount of iodine reduced by the starting flour, the weight fraction of each constituent expressed as percentage of the starting flour, the amount of iodine reduced by the constituent, and the fraction reduced by the constituent expressed as percentage of the iodine reduced by the starting flour. All values except those indicated were calculated on a dry-weight basis.

TABLE II
DISTRIBUTION OF IODINE-REDUCING CAPACITY AMONG THE CONSTITUENTS OF FLOURS OF VARYING BAKING QUALITY

WHEAT VARIETY	IODINE REDUCED	CONSTITUENT	WEIGHT FRACTION	IODINE REDUCED	FRACTION OF IODINE REDUCED
	μeq/g		%	μeq/g	%
Ponca	1.4	Gluten	13[a]	6.4[a]	61
		Starch	76	0.11	6
		Solubles	7	6.2	33
		Totals	96		100
Red Chief	1.7	Gluten	12[a]	6.8[a]	49
		Starch	76	0.09	4
		Solubles	7	10.6	47
		Totals	95		100
Golden Ball	1.8	Gluten	12[a]	8.9[a]	61
		Starch	78	0.11	5
		Solubles	9	6.7	34
		Totals	99		100
Lee	1.6	Gluten	12[a]	9.2[a]	69
		Starch	78	0.09	5
		Solubles	6	6.6	26
		Totals	96		100

[a] Based on protein (N × 5.7). Assuming the crude gluten contained 20% nonprotein constituents, the values for weight fraction of gluten, and consequently the total weight recovered, are approximately 3% low.

Values for the Ponca and Golden Ball varieties represent the average of duplicate determinations; those for Red Chief and Lee are based on two distinct separations of constituents and thus represent the average of two sets of duplicate determinations. The values for the separate preparations agreed within 5% or less.

Early titrations revealed that flour constituents separately reduced approximately 10% more iodine than the respective starting flours.

However, the high values were due to accumulation of salt in the soluble fractions as a result of washing the glutens with 0.1% sodium chloride. This salt is among the electrolytes found to cause high titers (see above). Therefore, the reported values for the soluble constituents are based on the difference between the amount of iodine reduced by the starting flour and the amount reduced by gluten plus starch.

The results in Table II for the hard red winter wheats indicate that the gluten of better quality reduced a higher fraction of the iodine reduced by the flour, despite the fact that the weight fractions of the constituents were nearly identical for both Ponca and Red Chief. A similar comparison can be made between the hard red spring wheat and the durum. Obviously, a greater number of flours must be investigated before any general conclusions can be reached regarding the differences observed. These differences appear to be of approximately the same magnitude as those reported in viscosity comparisons, but they are considerably smaller than those obtained by the Zeleny sedimentation test (18).

Values for the starting flours compare favorably with those obtained by the *o*-iodosobenzoate method of Nordin and Spencer (33) and by the recent amperometric method of Sokol, Mecham, and Pence (36). Values for the soluble fractions are higher than those reported by Baker, Parker, and Mize (2), but lower than those of De Lange and Hintzer (14).

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THE BREAD STALING PROBLEM. X-RAY DIFFRACTION STUDIES ON BREADS CONTAINING A CROSS-LINKED STARCH AND A HEAT-STABLE AMYLASE[1]

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ABSTRACT

The relationship between crumb firmness and starch crystallization of breads made with reconstituted flours containing 40% of a cross-linked starch and supplemented with a heat-stable bacterial amylase has been investigated. X-ray patterns showed that up to 3 or 4 days after baking, the experimental breads were more crystalline than a conventional bread. Extensive starch crystallization in amylase-supplemented bread was not paralleled, however, by a corresponding increase in crumb firmness.

X-ray patterns of isolated starch gels showed that the proportion of retrograded or "B" structure was greater in gels of the cross-linked starch than in comparable gels of unmodified wheat starch. Upon aging, the starch in either gel developed increased crystallinity irrespective of the presence of a heat-stable bacterial amylase. Breakdown of the network of crystalline and amorphous regions, resulting from amylase activity, can account for the lack of firmness in bread of high starch crystallinity.

Retrogradation of the starch component is the principal physical change that has been demonstrated to occur in bread during staling. Katz (10) was the first to show that the X-ray diffraction pattern of

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fresh bread was similar to that of freshly gelatinized wheat starch; whereas the pattern for stale bread was similar to that of retrograded starch. Patterns of retrograded starch were characterized by an increased number of lines, which showed that part of the starch had crystallized during retrogradation. Because the elastic moduli of high polymers generally increase with extent of crystallization (2), it may be reasoned that the firming of bread crumb which occurs during staling results from crystallization of the starch phase. Hellman, Fairchild, and Senti (6) measured the rate of crystallization of starch gels at moisture levels comparable to that found in bread. The curve found by these authors relating extent of crystallinity to age of the gel is similar to that relating crumb firmness to the age of bread, and it serves to support the assumption that crystallization of starch plays an important role in bread staling.

Prentice, Cuendet, and Geddes (13) found that bread made from reconstituted flour, in which part of the starch was replaced by a chemically cross-linked corn starch, had a firmer crumb than control loaves containing no cross-linked starch. In contrast, incorporation of bacterial alpha-amylase in the dough has been shown by several workers (5,9,11) to retard firming of the crumb. No studies were made, however, of the crystallinity of the starch phase in these breads. Recently, Bechtel (3) has investigated the effect of incorporation of a cross-linked corn starch and bacterial alpha-amylase, both singly and in combination, on the staling rate of bread as measured by taste-panel acceptance and by crumb firmness. These same breads were available for X-ray studies, and this paper reports relative crystallinities and rates of crystallization upon aging at selected levels of cross-linked starch and bacterial amylase.

Also reported are X-ray studies comparing unmodified wheat starch to the cross-linked starch for (a) original structure, (b) alteration of structure when heated at high moisture levels, and (c) retrogradation after gelatinization in the presence of bacterial alpha-amylase.

Materials and Methods

Materials. The cross-linked starch used was a commercial product sold under the trade name Vulca 100[2]. According to the manufacturer, the molecules in corn starch were cross-linked by reaction with epichlorohydrin[3]. The resulting starch retained its granular form but swelled only to a limited extent when heated in water. Gelatinization,

[2] National Starch Products Company, New York, N. Y. Mention of trade names or products does not constitute endorsement by the U. S. Department of Agriculture over others of a similar nature not mentioned.

[3] U. S. Patent 2,500,950.

as indicated by loss of birefringence, occurred at temperatures in the range 65° to 74° C., only slightly higher than the range 64° to 73°C. for unmodified corn starch.

Bread containing the cross-linked corn starch and bacterial alpha-amylase was baked at the American Institute of Baking, Chicago, Illinois (3), and was received at this laboratory within 18 hours after baking. One portion of each loaf was removed for immediate study, another portion was allowed to stale, and the remainder was quickly frozen at −14°C. to maintain it in a fresh state. Table I shows the composition of the breads studied. The selected breads were typical

TABLE I

COMPOSITION, CRUMB FIRMNESS, AND ACCEPTABILITY OF BREADS SELECTED FOR X-RAY DIFFRACTION STUDIES

Bread No.	Moisture Content	Enzyme Level	Cross-Linked Starch	Crumb Firmness (g. per sq. in.)			Taste-Panel Score[a]		
				Storage Time, Hours			Storage Time, Hours		
				2	20	140	2	20	140
	%	*SKB units/lb*	%						
3	36.6	24	40	5.1	5.1	5.3	5.6	5.4	4.5
4	36.6	4	40	5.1	6.6	8.5	5.2	4.6	3.1
2	35.0	24	40	5.1	6.6	8.5	5.4	5.0	3.4
1	35.0	24	0	2.9	4.8	6.4	5.9	5.3	4.4

[a] Rating scale units: 6, very fresh; 5, fresh; 4, slightly fresh; 3, slightly stale; 2, stale; 1, very stale.

of those examined in Bechtel's experiments (3); composition variables included water content, the incorporation of enzymes, and the addition of a cross-linked starch. Reconstituted flours were used, and with the exception of bread No. 1, 40% of the wheat starch was replaced by the modified starch.

Methods. Bread slices wrapped in waxed paper and enclosed in air-tight plastic bags were permitted to stale at room temperature. X-ray patterns were taken after 1, 3, 5, 8, and 12 days' staling subsequent to baking. The X-ray sample mounts were prepared by packing the crumb into a chamber (1.5-mm. diameter by 1 mm. thick) which was sealed with windows made from 0.00025-in. Mylar film, type A. This material did not interfere with the transmission of X-rays, but it prevented moisture loss during the exposure.

X-ray patterns were taken with nickel-filtered, CuK-alpha radiation at 35 kv. and 15 ma. for 1.5 hours. To provide clear registration of weak diffraction lines, the space between sample and film was enclosed and filled with helium. Sample size, time of exposure to X-radiation, and film development were carefully controlled so that patterns would be comparable.

Starch gels, at water concentrations similar to those of bread doughs, were prepared by the techniques described by Hellman *et al.* (6). The bacterial alpha-amylase incorporated into the starch gels was from the same lot used in the bread-baking experiments and was in the form of a dry powder. After enzymatic activity was determined, by the method of Sandstedt *et al.* (1,14), the enzyme was suspended in water and added to the starch in aliquots calculated to give desired levels of activity.

Results

X-Ray Diffraction Patterns of Bread Crumb. Figure 1 is a schematic diagram of the X-ray patterns referred to in the text and follows the established nomenclature for starch patterns. The dotted lines indicate weak lines that may not always be observed in patterns of poorly

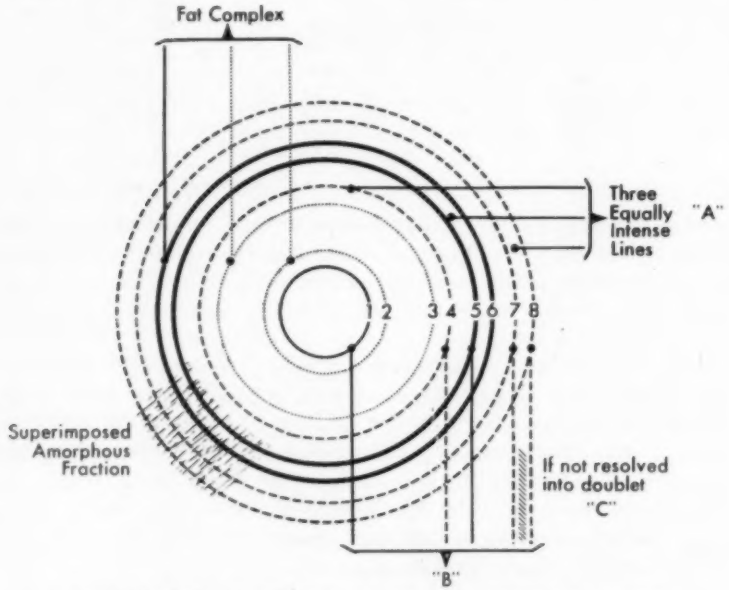

Fig. 1. Schematic diagram of X-ray patterns associated with starches.

crystallized samples, such as freshly prepared bread or gels. The "A" diagram is given by the crystalline structure normally found in raw cereal starches. During baking this structure is lost, and subsequent recrystallization of the starch takes place into structures which give

patterns designated in Fig. 1 as "B," "C," or starch-fat complex. X-ray patterns show the "B" type of crystallinity occurs in aged bread and in retrograded starch gels (6,10). Patterns of starch gels often show additional lines which Katz (10) ascribed to the V structure of starch. Hellman *et al.* (6) show that this pattern is produced by a starch-fat complex rather than by starch alone.

Patterns of bread are described in terms of their characteristic lines. Diffraction patterns of breads numbered from 1 to 4 (Table I) after 1 day of aging consisted of lines 1, 5, and 6. If reheated to refreshen, only lines 5 and 6 remained. Patterns of these breads after 5 days had line 6 of the starch-fat complex and, in addition, all the lines of the "B" structure. Conventional bread baked from whole flour without added bacterial alpha-amylase differed from the experimental breads (Table I) in that the day-old bread was weaker in the "B" structure, as shown in Fig. 2. Moreover, both oven-fresh and an aged conven-

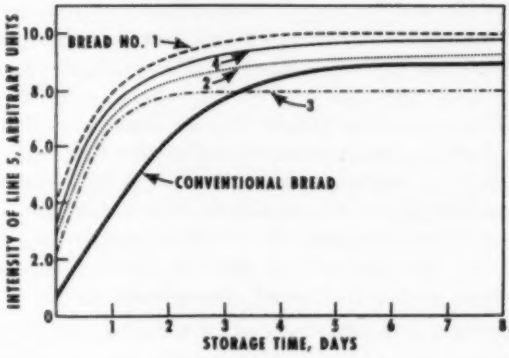

Fig. 2. Relative extent of "B" type of starch crystallinity in breads at 25°C.

tional bread refreshened by heating had line 6 as the only prominent line together with a very weak line 5.

Relative degrees of starch crystallinity in fresh and aged breads were estimated from the intensity of line 5 (Fig. 1). Extent of crystallinity determined in this manner for breads varying in type of starch and in amount of bacterial alpha-amylase is compared in Fig. 2 with the crystallinity of starch in conventional bread as a function of aging. Up to 3 or 4 days, starch in breads 1 to 4 had crystallized to a greater extent than had the starch in conventional bread. The crystallization behavior of bread 4 showed the effect of replacing 40% of the wheat starch with cross-linked starch. Bread 3 crystallized rapidly dur-

ing the first day, but upon continued storage it remained at nearly the same level of starch crystallinity. Data in Fig. 2 on bread 1 indicate that the addition of enzyme promoted starch crystallization. This bread, baked from a reconstituted flour which contained no cross-linked starch, would be expected to be most susceptible to the action of bacterial amylase. If degradation of starch by alpha-amylase hindered crystallization, as might be expected on the basis of the slightly gummy character of the crumb, bread 1 should have given X-ray patterns indicative of low crystallinity. Quite the opposite effect was observed; crystallization was not hindered, and this bread consistently had the greatest extent and perfection of crystalline regions.

Since breads listed in Table I were 18 hours old when received, their crystallinity in the oven-fresh condition could not be determined directly and compared with conventional bread baked at this laboratory. It was found, however, that day-old conventional bread, or defrosted conventional bread that had been frozen immediately after baking, gave the same X-ray pattern as the oven-fresh bread after it was heated for 30 minutes at 95°C. in sealed containers. Retrograded gels of cross-linked and unmodified starches also gave X-ray patterns identical to those of fresh gels after a similar heat-treatment. The assumption was made that breads from the reconstituted flours would behave similarly, and their crystallinity at zero time was determined from X-ray patterns taken after reheating under these conditions. Each experimental bread gave a more intense line 5 than the conventional bread at zero time; this indicated a greater proportion of the starch was in the "B" structure, and, in fact, the extent of crystallinity was about the same as that in day-old conventional bread.

Intensity changes in other lines of the X-ray patterns were consistent with the changes in starch crystallinity already described. Although increase in the intensity of line 1 (16Å spacing) may appear ideal as a measure of "B"-type crystallinity, it was complicated by a sensitivity to moisture loss, tending to decrease in intensity on slight reduction in water content. This sensitivity, however, may be the basis for interpretation of the observation that enzyme-containing breads exhibited a greater stability than conventional bread toward loss of intensity of the 16Å line under the same storage conditions. This difference would indicate that the starch component in breads with added enzyme retained a greater amount of water on aging and therefore should have been better plasticized and less rigid than the starch component of a conventional bread of equal crystallinity. This does not necessarily imply that the enzyme-supplemented loaves lost less moisture on aging,

but only that less moisture was transferred from the starch to other components of the bread.

Starch Gels. The cross-linked starch at 10% moisture content gave the "A" type of X-ray pattern (Fig. 1). This pattern demonstrated that little molecular disordering occurred during manufacture and that these starches were similar to unmodified cereal starch in structure, except for the restraints imposed by the cross-links between adjacent chains.

About 46% water was required to form a coherent gel as compared with 43% water required for gel formation in an unmodified wheat starch (6). A diffraction pattern of the cooled fresh gel showed that, apart from amorphous material, any structure present was predominantly "B" type; this was indicated by strong but diffuse lines in the pattern corresponding to those labeled 4 and 5 in Fig. 1. Line 1 was evident as a faint diffuse ring. In contrast, the discrete lines in the pattern of freshly baked wheat-starch gels were those of the starch-fat complex. The starch-fat complex was present, and its amount was judged mainly by the intensity of line 6 (Fig. 1), although lines 2 and 3, which are characteristic of the complex, were also present on the patterns.

Mixtures of cross-linked corn starch and wheat starch, when baked at water contents sufficient for gel formation, gave patterns that showed the presence of both "B" and starch-fat complex structures. The patterns duplicated those given by breads of similar composition. Patterns of 1-day-old gels containing the starches singly or in combination had well-developed 16Å lines; on subsequent aging, the gel showed increased clarity and intensification of the "B" pattern. Intensity of the lines due to the starch-fat complex did not increase during storage of the gels for 1 week.

Effect of bacterial alpha-amylase on starch crystallization was investigated with gels which contained either wheat or cross-linked starches. Wheat starch gels at 45% and cross-linked corn starch gels at 46.5% water content were prepared and aged at enzyme levels equivalent to 0, 4, 24, and 96 SKB units per lb. To obtain estimates of the degree of crystallinity, X-ray patterns of the gels were judged in the manner prescribed for bread. The results closely paralleled those observed for the breads. Gels with no added enzyme, for example, crystallized more slowly than those with added enzymes. While only slight effects were noted at the 4 SKB level, gels containing 24 and 96 SKB units per lb. of bacterial alpha-amylase appeared after 1 day of aging to reach a level of crystallinity that remained practically unchanged for at least 4 days. At that time, all gels were remarkably strong in

"B"-type crystallinity, and only the wheat starch gel at the 96 SKB level showed a slight decrease in amount of crystallized starch. Although starch crystallization was practically unhindered, certain gels were altered in other physical properties by the addition of bacterial alpha-amylase. The wheat starch gels at the two highest enzyme levels, for instance, were much softer and more pliable than the control gel; moreover, the aged gels did not crumble when rolled or pressed onto a glass slide.

Discussion

Physical properties and taste-panel acceptance of breads containing a cross-linked starch and bacterial alpha-amylase are reported by Bechtel (3).

Selected data from that report are given in Table I, which includes 2-hour and 140-hour firmness values and the taste-panel ratings of staling or acceptability for breads selected for the X-ray studies reported in this paper.

Crystallization and Staling. Breads containing cross-linked starch and bacterial alpha-amylase were more crystalline than conventional bread with neither enzymes nor modified starch, both as freshly baked (refreshened as described above) and for 3 to 4 days after baking. From the observations on starch gels, there were two apparent reasons for this: 1) a fresh cross-linked starch gel was more crystalline than a comparable wheat starch gel, and 2) starch crystallization appeared to proceed at a faster rate in fresh gels at certain levels of alpha-amylase. Both of these factors contributed in breads 2, 3, and 4, while only the second one contributed in bread 1. The relatively high starch crystallinity in the breads in the fresh state can account for the high initial firmness values of these breads, which are listed in Table I.

Firmness and acceptability as given in Table I clearly were not sensitive to changes in starch crystallinity which occurred in breads containing added alpha-amylase. The considerable increase in crystallinity (Fig. 2) that developed in bread 3 during the first day, for example, was not accompanied by correspondingly large changes in firmness and acceptability. This bread remained at a high level of starch crystallinity on aging but retained its initial softness and showed the least decrease in acceptability. Bread 2, which had developed an extensive degree of starch crystallinity at 140 hours, still retained a fair degree of acceptance. Bread 1, in particular, did not follow the expectation that enzymatic action results in less starch crystallization in the crumb. As described under the heading "Results," this bread exhibited exceptional crystallinity. Contrary to what would be ex-

pected of a crumb having a high degree of starch crystallinity, the firmness of the crumb at 140 hours was relatively low. Moreover, No. 1 was exceeded in taste-panel acceptability only by No. 3, the bread estimated to have the least proportion of crystallized starch. Bread 4, at one-sixth the enzyme level of the other breads, had lower acceptability throughout the aging period although it differed little in crystallinity.

It has been suggested that decreased starch retrogradation accounts for the benefits incurred when breads are supplemented with bacterial alpha-amylase. Evidence for decreased retrogradation in the enzyme-supplemented bread has been the relatively small decrease in proportion of solubles extractable from the aged crumb and the relatively high susceptibility of its starch component in stored breads to enzyme degradation (7,8,12,15). Retention of crumb solubility of the starch and susceptibility to enzyme attack on aging, however, do not preclude crystallization. It is concluded from the present work that starch crystallization was not significantly hindered by the incorporation of bacterial alpha-amylase at levels which softened the crumb. In the case of starch gels, crystallization was decreased only at enzyme levels greatly in excess of those used in the breads. There also was evidence that, at certain levels, starch crystallization was enhanced.

Although our results and those of others show a good correlation between firmness and degree of crystallinity in conventional bread, it is clear from the results on breads supplemented with bacterial amylase that increased crystallinity alone is insufficient to produce a firmer crumb. Because formation of crystalline regions in amorphous, freshly gelatinized starch causes the starch structure to become more rigid, changes introduced into this structure by the action of alpha-amylase also must account for the failure to develop a rigid structure in bacterial alpha-amylase supplemented breads.

Figure 3 is a schematic representation of the network of crystalline and amorphous regions that likely exists in a partially crystalline high-

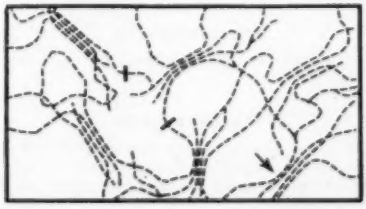

Fig. 3. A diagrammatic representation of a network of crystallites and intervening amorphous regions.

polymer material. The arrow points to a crystallite formed by the association of chains. Openness of the structure in the amorphous regions is exaggerated for the purpose of illustration. The distribution of X-ray intensity between the discrete line pattern arising from crystalline regions in starch and the background scattering due to amorphous regions provides good evidence that this schematic representation is applicable to starch. A variety of structures may exist in the amorphous regions. Generally, these regions are characterized by lower density, fewer contacts between adjacent molecules, and greater accessibility to chemical reagents.[4] A given polymer chain may pass through several crystalline regions, and a given crystalline region may contain polymer chains which pass through several different crystalline regions. Crystalline regions thus serve as tie-points which restrict the movement of the polymer chains when subjected to stress and, in this manner, contribute to the rigidity of the polymer structure.

It is apparent, however, that scission of chains in the amorphous regions will tend to free the structure of the restraints imposed by the crystalline regions. In the case of starch, hydrolytic cleavage of the chains by bacterial alpha-amylase would be expected to occur preferentially in amorphous regions because here the structure is most open and accessible to attack. Such chain scission in the amorphous regions, as indicated by the two cross-bars in Fig. 3, would give the crystallites greater freedom to move independently of one another with a resulting decrease in rigidity of the system. This effect should be most marked at high moisture contents (e.g., in bread) where slippage of starch molecules past each other is facilitated by the plasticizing action of the water molecules.

The greater crystallinity on aging observed in starch gels at certain levels of bacterial amylase activity may also result from chain scissioning in the amorphous regions. The resultant freedom of motion given to chain segments near crystallites would enable these segments to align themselves properly for crystallization. A similar increase in crystallinity has been observed in cellulose after light acid hydrolysis (4).

Acknowledgment

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THE CONTRIBUTION OF THE GERM TO THE OIL CONTENT OF WHITE FLOUR[1]

D. J. STEVENS

ABSTRACT

A sample of No. 2 Manitoba Northern wheat freed from germ by insect attack was laboratory-milled comparably with the control wheat. The two resulting straight-run flours gave similar baking results, except that the loaf from the germ-free sample had much the paler crust. Comparison of oil contents in corresponding flour streams indicated that in the control samples the following percentages of the oil contents originated from the germ: straight-run flour, 27; break flour, 24; flour from small middlings or dunst reduction, 23–24; flour from sizings (semolina) reduction, 34. These amounts were contained in (a) fragments of germ present in the flour and (b) endosperm particles to which oil had been transferred by expression from germ particles during reduction rolling. In the break flour, the germ oil contribution was mainly from (a); in the break middlings and reduction middlings (dunst) flours, mainly from (b); in the sizings (semolina) flour, from both (a) and (b). The straight-run flour contained in all, in forms (a) and (b), 30% of the oil initially present in the germ of the wheat. Its scutellum content was roughly 0.3%.

Values for the oil content of flour, bran, and germ have been determined by many workers who have used the milling products, but, as Horder, Dodds, and Moran (10), have pointed out, these are not pure anatomical parts of the wheat grain. Each consists of a mixture of endosperm, bran, and germ, with one component dominant. In the case of flour, a small amount of bran is present (though the miller's aim is to minimize this), together with an appreciable amount of the scutellum portion of the germ, which makes an important contribution to the thiamine content of the flour. The behavior of the scutellum during white flour milling was studied by Kent, Simpson, Jones, and Moran (13), who found that one-third of it remained attached to the bran during breaking, the remainder being found in stocks released from the second break onwards. During the second break, it was released chiefly into the sizings (coarse semolina)[2]; thereafter an increasing proportion of fine particles was produced. The scutellum was liberated from English wheat earlier in the breaking system than from Manitoba, and in both cases, earlier liberation resulted from reducing the moisture content of the millfeed.

Further work by Kent, Thomlinson, and Jones (14) on the effects of tempering showed that, in the case of a mixed grist at 14.3% mois-

[1] Manuscript received November 21, 1958. Contribution from the Research Association of British Flour-Millers, St. Albans, England.

[2] Where the English milling term differs from the American, it is given in parentheses throughout this paper.

ture content, the early breaks released a 30% yield of the grade of sizings (semolina) sifted between Nos. 20 and 40 grits gauzes and contained about three-quarters of the total embryo in the wheat and one-fifth of the scutellum. Increasing the moisture content of the millfeed to 17.3% reduced the corresponding proportions of embryo and scutellum to less than half of those at 14.3%.

Stocks from the early breaks were found to contain embryo fragments throughout all particle size fractions, but scutellum fragments were concentrated in the coarser fractions. The reduction of coarse stocks caused almost all of the embryo content to be flattened, as well as much of the scutellum. In the later reductions, however, finer particles of scutellum present in the feeds to the rolls were more readily pulverized. The behavior of both embryo and scutellum during smooth roll reduction depended on particle size, on moisture content, and on earlier mechanical treatment in the milling process.

The flaked germ produced during reduction is, of course, removed by sifting, but oil expressed from the germ during flake formation may be transferred to endosperm particles and so enter the sifted flour. The extent of loss of oil from the germ by expression is considerable. The oil content of commercial germ, which consists chiefly of embryo flakes, ranges from 6 to 11% (4); whereas hand-dissected embryo has an oil content of 15% (8).

The present investigation was undertaken to determine to what extent germ oil occurs in white flour, not only because of the presence of germ particles, but also as a result of the expression of oil during flaking.

Materials and Methods

Preparation of Germ-Free Wheat Sample. Laborious mechanical methods such as the use of a dental drill (16) were considered to be impracticable for the preparation of a sufficient quantity of germ-free wheat for laboratory milling. It is well known, however, that several insect pests remove germ effectively, and it was found that the adult confused flour beetle (*Tribolium confusum* Duv.) was particularly convenient, as no webbing was formed, and the frass was readily removed by sifting. Satisfactory breeding was carried out in jars of wholemeal flour incubated at 30°C. and 80% r.h. Adult beetles were removed by sifting, and batches of 100 g. of a No. 2 Manitoba wheat were then incubated with approximately 2,000 beetles for 10 days. The germ-free grain was then sorted out and the beetles removed by a suction probe, and a yield of about 80% was usually obtained. Examination under low-power magnification showed that the embryo

and scutellum were satisfactorily removed. Longer exposure to attack did not increase the yield, since the beetles then attacked the endosperm despite the presence of intact grains.

Laboratory Milling. In order to avoid direct entry of water into the exposed endosperm at the site of germ removal in the germ-free wheat, a special tempering procedure was adopted. Small samples at a time were shaken on a flat, damp surface, so that only the bran was wetted and the concave exposed surface of the endosperm did not come into contact with moisture. A flat, endless rubber belt was driven on horizontally mounted rollers, and water was applied from a rotating brush held against one end of the belt. Fifty-gram batches of wheat were tipped on to the surface and covered by an inverted glass dish, which was then moved briskly to and fro so that the grains were thrown about on the damp surface. The time necessary to increase the moisture content to 16½–17% was determined on trial samples.

Both the germ-free and the control samples were damped in this way and stored in stoppered jars overnight. They were then milled side-by-side at each stage under identical conditions to about 70% extraction, by means of the open laboratory-milling system (3) with four breaks. On the reduction side, four reductions of sizings (semolina) were made, and the reduction middlings (dunst) produced were in turn given three reductions. In normal practice, the reduction middlings (dunst) are combined with the break middlings before reduction since both materials have similar granularity. Since their compositions are different, especially as to germ content, they were treated separately in the present work.

Shortage of material made it impossible to examine the 14 flour streams separately, so they were grouped as follows: 1), break flour; 2), flour from reduction of break middlings; 3), flour from reduction of sizings (semolina); and 4), flour from reduction of reduction middlings (dunst). The coarse bran and the offal removed during reduction of sizings (semolina) were also examined; the latter was particularly rich in germ in the case of the control sample.

Samples of cleaned bran were prepared by agitation on a No. 36 grits gauze loaded with small rubber balls, until no more dislodged endosperm particles passed through the sieve.

Intact germ was hand-picked from the coarse sizings (coarse semolina) produced on passage of the dry wheat through coarsely fluted break rolls (8 cuts per in.). It consisted mainly of the embryo with a little attached scutellum and was not at all flattened.

Microscopic Detection of Germ Particles in Flour. Several staining methods were tried in attempts to identify and estimate germ particles.

Although none was completely satisfactory, a brief account may be of interest.

Mucicarmine gave a good uniform red stain with germ particles, but aleurone cell contents were also stained.

The Crystal Violet technique of Larkin, MacMasters, and Rist (15) was found to be very satisfactory for separating the stained bran and germ particles from flour. With some practice it was found to be possible to distinguish between the bran and germ because of the following differences: 1), germ particles not so intensely stained as bran; 2), germ particles rounded in shape with ill-defined outline, bran particles angular and well defined; 3), germ particles jellylike on probing with a needle, bran particles rigid; and 4), higher magnification showed up fine structure of particles. Identification of germ particles could thus be confidently carried out, but the method was unsuitable for quantitative work because of its tedious nature and also because of the wide range of particle sizes which was found to occur.

The methoxy hydroquinones which occur in the germ give a blue color with 2:6 dichloroquinone-4-chloroimide (5). An alcoholic solution of this material was tried as a spray reagent with samples of flour lightly dusted onto filter paper. The color was developed by holding over ammonia, after which the sample was examined under low-power magnification. The endosperm gave a pale-pink background color resulting from decomposition of the reagent, against which the intense-blue germ particles showed up very clearly. However, although bran particles and aleurone cell contents gave no appreciable coloration, results were rather unsatisfactory because of considerable variation in the intensity of the blue color of the germ. A colorimetric method using the DCQ reagent with aqueous extracts of flour samples has, however, been developed by Daniels (6) for estimation of the methoxy hydroquinone content. His results show that the concentration in the scutellum is very great, so that it is almost certain that the blue specks given by the spray reagent on dry samples were due to scutellum alone.

Oil was extracted with boiling petroleum ether (b.pt. 40°–60°C.), in the Bolton and Revis apparatus (17). Residual solvent was removed from the extracted oil by oven-heating at 100°C. for 40 minutes; the extraction flask was flushed with air occasionally during the first 10 minutes to remove vapor. All samples were given identical treatment in view of the changes in the extracts which can occur on heating (7). Refractive index measurements on the extracted oil were made at 20°C. with an Abbé refractometer.

Thiamine was estimated fluorimetrically by the thiochrome method.

Nitrogen was estimated by the standard Kjeldahl method, with the factor 5.7 used for conversion to protein content.

Phosphorus was estimated colorimetrically as the blue reduction product of phosphomolybdic acid (2).

Results and Discussion

Milling Yields. The yields of products from the milling of the control and germ-free wheats were:

	Control %	*Germ-free* %
Flour	69.7	72.0
Fine offal	16.1	14.7
Bran	14.2	13.3

These results show a higher apparent extraction rate in the case of the germ-free wheat, together with a lower yield of offals. This effect was observed by Aitken (1) on a sample of wheat damaged by larvae of the Indian meal moth. If the proportion of germ is 3%, the yield from the germ-free sample would be 69.7/(100−3) or 71.9%, in good agreement with the observed figure.

Baking Tests. Extensimeter results and water absorptions were closely similar for both straight-run flours. Small-type loaves were baked from them by a method using 1.1% yeast, 1.4% salt, 3 hours' bulk fermentation at 80° F. (26.6°C.), and 60 minutes' final proof. The control flour gave a loaf typical of a 70%-extraction Manitoba flour, and the germ-free sample gave a loaf closely similar in volume, crumb color, and texture. This contrasts with Aitken's finding (1) that flour from germ-free wheat gave an inferior loaf. The only definite difference observed in the present case was that the loaf from the germ-free sample had a very pale crust.

Analytical Results. Table I gives the oil, protein, thiamine, and phosphorus contents (expressed at 14% moisture content) of milling products from both the control and germ-free wheat, together with the refractive index of the extracted oil at 20°C.

The thiamine content of the intact germ sample corresponds to a scutellum content of about 8%. The refractive index of the germ oil is low compared to that of bran and endosperm. Each of the samples derived from the control wheat has higher oil, protein, thiamine, and phosphorus contents than has the corresponding sample from the germ-free wheat, and the oil has a lower refractive index. Very large differences are shown in the offal from sizings (semolina) reduction, since the control sample contains a high concentration of germ. The figures for the bran samples show that the germ present in that from the con-

TABLE I

ANALYTICAL RESULTS[a] ON MILLING PRODUCTS FROM CONTROL AND GERM-FREE WHEATS

SAMPLE		YIELD	OIL[b]	REFRACTIVE INDEX OF OIL n_D^{20}	PROTEIN (N × 5.7)	THIAMINE	PHOSPHORUS	GERM OIL CONTENT	
		%	%		%	γ/g	mg/g	g/100 g sample	g/100 g oil
Break flour	Control	17.4	1.18	1.4777	15.7	0.73	1.62	0.28	24
	Germ-free	18.6	0.90	1.4781	15.3	0.50	1.50	...	..
Flour from reduction of:									
Break middlings	Control	10.6	1.09	1.4788	13.3	0.97	1.57	0.25	23
	Germ-free	12.3	0.84	1.4795	13.0	0.44	1.49		
Sizings (semolina)	Control	23.5	1.27	1.4791	12.15	0.95	1.39	0.43	34
	Germ-free	24.2	0.84	1.4801	11.6	0.32	1.30	...	..
Reduction middlings (dunst)	Control	18.2	1.04	1.4807	11.45	0.82	1.07	0.25	24
	Germ-free	16.9	0.79	1.4811	11.35	0.25	1.00	...	..
Total straight-run flour	Control	69.7	1.16	1.4789	13.0	0.85	1.40	0.31	27
	Germ-free	72.0	0.85	1.4797	12.75	0.38	1.32	...	..
Coarse bran	Control	13.1	3.84	1.4771	13.55	6.63	...	0.90	24
	Germ-free	12.6	2.94	1.4780	13.45	4.81	...	...	..
Offal from reduction of sizings (semolina)	Control	11.1	4.17	1.4764	15.7	15.58	...	2.37	57
	Germ-free	10.6	1.80	1.4788	12.55	2.56	...	...	..
Cleaned bran[c]	Control		4.70	1.4784					
	Germ-free		3.70	1.4791					
Intact germ[c]			17.0	1.4760	....	22.0			

[a] Results expressed on 14% moisture basis.
[b] By petroleum ether extraction.
[c] See text under "Laboratory Milling."

trol wheat has not been entirely removed by the cleaning process described earlier. The refractive index of the germ-free bran oil is similar to that from the straight-run flour, so that the presence of bran in flour samples does not greatly affect refractive index values.

The difference in oil contents for all corresponding pairs of flour samples is quite marked, which shows that a significant amount of the oil content of the control samples must originate from the germ; the figure for the straight-run flour is 27% of its total oil content. From Hinton's data (8), the total germ oil content (from both embryo and scutellum) of the whole grain may be calculated to be about 0.7%. Since 143 parts of grain yield 100 parts of 70%-extraction flour, the oil content of such flour would be about 1% higher if all of the germ oil entered than if none did so. The actual measured difference of 0.31% between the oil contents of the straight-run flours from the control and germ-free wheats thus indicates that about 30% of the total germ oil initially present in the control wheat has, in one form or another, entered the flour milled from it.

The flour obtained from the germ-free sample on rolling the reduction middlings (dunst) is particularly interesting since it represents virtually pure endosperm, free not only from germ particles but also from transferred germ oil. It therefore has the lowest oil, protein, thiamine, and phosphorus contents of all the samples, and its oil has the highest refractive index. Jacobs and Rask (11) calculated the oil content of the inner endosperm to be 0.75%, and this is supported by the present value of 0.78%. The thiamine content of 0.25γ per g. is very low and may be compared with the values reported by Hinton (9) for dissected endosperm, which ranged from 0.1γ per g. for the central zone to 0.3γ per g. for the outer.

The amount of germ oil derived from the transfer effect could be calculated from the total germ oil content by subtracting the contribution from germ particles. This, however, would require the estimation of particles of embryo and scutellum separately because of their different oil contents of about 15% and 30%, respectively. Since microscopic staining methods failed to give a measure of the actual amounts of embryo and scutellum in the control flour samples, an attempt was made to calculate these from analytical data for corresponding pairs of control and germ-free samples. Thiamine and phosphorus contents were chosen for this purpose, since both are concentrated in the germ, and the values for embryo and scutellum have been reported by Hinton (8). Unfortunately, the use of simultaneous equations involving small numerical differences in the analytical data gave results which were too inaccurate to be of quantitative use. In all cases, however, the

values for the germ particle content of control samples were too small to account for the differences in oil content between control and germ-free samples, indicating the presence of germ oil other than that contained in germ particles. An estimate of the amount of oil lost from embryo particles during commercial flaking was made by measuring the oil content of embryo flakes hand-picked from commercial germ. A value of 6.8% was obtained, compared with 15.4% for dissected embryo. Transfer of the expressed oil to endosperm would increase the oil content of a 70% flour by about 0.15% (taking the embryo to comprise 1.3% of the grain weight), which represents about half the total germ oil found in the laboratory-milled control flour. The intensity of the flaking produced by the laboratory milling was, however, much less pronounced than in commercial practice.

The difference in thiamine content between the control and germ-free straight-run flours is due mainly to the presence of scutellum in the control sample. Taking the thiamine content of the scutellum to be 170γ per g. (8), a scutellum content of about 0.3% is indicated.

The difference in behavior of the embryo and scutellum is illustrated by the analytical results on corresponding pairs of flour samples from the control and germ-free wheats. During passage through the break rolls, both portions of the germ become cut into particles which concentrate chiefly in the sizings (semolina) and break middlings fractions. In the later breaks, however, some particles of flour fineness are produced, though this would appear to occur mainly in the case of the embryo since the thiamine content is not high, especially in relation to the poor color-grade value of break flour (12). The break middlings fraction has a considerable scutellum content, which is sufficiently friable to give the flour produced on reduction a higher thiamine content than that of the break flour. The embryo particle content, on the other hand, is lower, as a result of the reduction rolling and sifting. The sizings (semolina) fraction is particularly rich in germ particles, and the flour produced from it has the highest oil content of the four groups. This oil is derived partly from the germ particles which it contains, particularly scutellum as indicated by the thiamine level, and partly by transfer of oil from the embryo flakes which become sifted out in the offal after loss of oil by expression. Oil will be transferred not only to the finest endosperm particles produced on reduction, but also to the intermediate particles or middlings (dunst). This fraction, therefore, already contains germ oil owing to the transfer effect, and in this respect differs from the break middlings. Its composition is somewhat similar to that of the sizings (semolina) reduction flour but in a larger particle size range. During the heavy reduction rolling

which it undergoes, powdering of scutellum particles down to flour fineness gives a considerable thiamine content to the flour, while embryo particles become extensively flattened and sifted off and lose oil to the flour in the process. Thus, although the flour obtained by rolling the reduction middlings (dunst) is equivalent to the highest grade patent flour and is often considered to consist almost entirely of endosperm, comparison of the results for the control and germ-free products clearly shows the presence of appreciable amounts of germ oil and of scutellum. Since the embryo particle content of this flour is very low, the difference in thiamine values between the control and germ-free samples may reasonably be attributed to scutellum, and a scutellum content of 0.35% is then indicated. This amount will contribute 0.1% of germ oil to the flour, the remaining 0.15% being derived from the transfer effect.

The relative amounts of germ oil from the two sources vary from one flour stream to another and also depend on the particular milling system used. The results of the present investigation are therefore limited to the laboratory milling system employed, which had no treatment comparable to commercial-scale purification of the break stocks prior to reduction. The operation of purifiers was found by Kent, Thomlinson, and Jones (14) to have a marked effect on the scutellum content of the feeds to reduction rolls since much was diverted to offal via the purifier tails. In this respect, therefore, the extent to which germ particles and expressed germ oil enter flour was increased in the present laboratory-milled flours. On the other hand, the intensity of flaking produced by the laboratory reduction rolling was far less than that which occurs in commercial practice, and also the moisture content of the mill feed was higher than might sometimes be employed.

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NOTE ON A RAPID METHOD OF DETECTING GERM DAMAGE IN WHEAT AND CORN[1]

Clyde M. Christensen and Subhi A. Qasem[2]

Of the several types of damage that influence grade designations of wheat and corn in commercial channels, the dark discoloration of the germ or embryo commonly known as "sick" wheat or, in corn, simply as "germ damage" is the most difficult to detect. In some cases of germ damage the entire kernel may be discolored, but usually this occurs only in the last stages of deterioration. During the past several years the writers have examined some hundreds of lots of wheat from commercial bins in many parts of the United States which contained from 0 to 80% "sick" or germ-damaged kernels. The great majority of these looked perfectly sound when examined with the unaided eye or with a

[1] Manuscript received July 28, 1958. Paper No. 3962, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota.
[2] Professor and Research Assistant, respectively, Department of Plant Pathology, Institute of Agriculture, University of Minnesota, St. Paul 1, Minnesota.

stereoscopic microscope under magnification of ×10 or ×20. In all of these, it was necessary to remove the pericarp from the germ to determine whether the germ was sound or damaged. In practice, inspectors examine kernels one by one, removing the pericarp from above the germ with tweezers. The process is time-consuming and tedious, especially when some of the kernels are small or shriveled, and any method which would make such inspection easier and faster without sacrifice of reliability would be of some practical value. The method here described has been used for some time in laboratory examination of samples of grain from commercial storage, and it appears to give rapid and reliable evaluation of germ damage.

Method. Four hundred kernels are counted out, placed in a beaker, 2% sodium hypochlorite sufficient to cover the grain added, heated to boiling, and boiled until the kernels begin to whiten. The hot liquid is poured off, the grain flushed twice with cold water, grain and water poured into a flat-bottomed dish and examined with the naked eye. The dark germs are readily apparent, whereas the sound germs are white or pale tan as shown in Fig. 1. With wheat, agitation of the dish while the kernels are being examined permits the germs of all kernels to be seen; with corn, it may be necessary to turn the seeds over to bring the embryo side uppermost. The kernels with dark germs are removed and counted. Although in commercial practice the percentage of germ-damaged grain is determined by weight, in senior author's experience (1) determination of amounts of "sick" wheat by

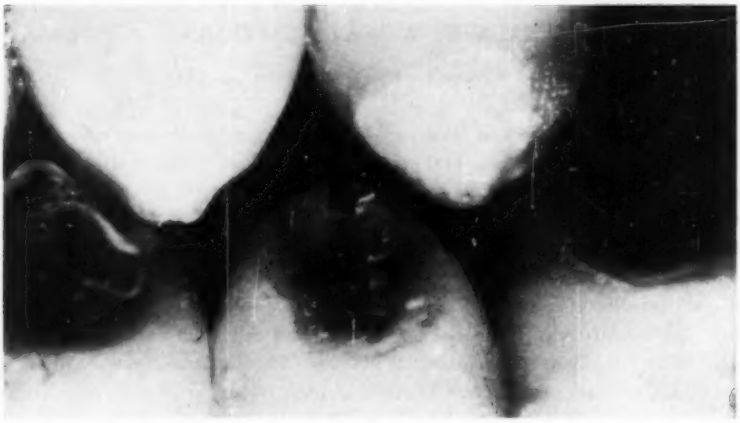

Fig. 1. Sound and germ-damaged or sick seeds after bleaching with sodium hypochlorite as described in the text. Top, sound germs; bottom, damaged germs.

count and by weight gave essentially the same results in nearly all cases; and it would seem that the use of numbers of germ-damaged seed rather than weight is a valid procedure.

Usually only 10 to 15 minutes, at most, are required to determine the amount of germ damage in a given sample. Intergrades between the dark germs typical of "sick" wheat and the light-colored germs typical of sound kernels, offer no more difficulty with this bleaching method than with the conventional procedure. In our laboratory examination of germ damage in corn an intermediate group, designated "ochre germs," has been used; the germs so designated are not dark enough to be rated as damaged by commercial grain inspectors, but they usually are heavily invaded by storage molds, are dead, and are in the intermediate stages between sound and damaged.

Typical results of examination of samples of wheat and corn by the conventional method and the bleaching method are given in Table I.

TABLE I

AMOUNT OF GERM DAMAGE IN COMMERCIAL SAMPLES OF WHEAT AND CORN AS DETERMINED BY CONVENTIONAL INSPECTION METHOD AND BY THE BLEACHING METHOD

WHEAT		CORN			
Conventional[a] Method	Bleaching Method	Conventional[b] Method		Bleaching Method	
Germs Dark	Germs Dark	Germs Ochre	Germs Dark	Germs Ochre	Germs Dark
%	%	%	%	%	%
1.0	1.0	0.5	0.0	1.5	0.0
1.5	1.0	1.0	1.0	1.25	0.5
2.9	3.3	2.0	1.0	1.75	2.0
3.4	3.0	4.0	0.0	3.0	0.75
6.0	6.0	8.0	0.0	5.0	1.0
6.2	8.3	10.0	10.75	11.0	11.0
8.0	9.3	12.0	5.0	13.0	3.0
8.5	8.0	19.25	4.24	17.5	2.0
24.0	25.7	22.5	9.0	21.75	9.0
45.0	43.0	38.5	15.75	44.0	12.0

[a] Determinations by Federal grain inspectors.
[b] Determinations by the writers.

Variations as great as, or greater than, these are not uncommonly encountered by different inspectors dealing with replicate samples from a given parcel of grain.

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ERRATUM

Cereal Chemistry, Vol. 36, No. 4
July, 1959

R. L. GLASS, J. G. PONTE, JR., C. M. CHRISTENSEN, and W. F. GEDDES:
Page 343. In paragraph 7, last sentence should read: "The results are expressed . . . from 100 g. dry wheat."
Page 345, Fig. 2, and page 352, Fig. 6. The legend for the vertical axis should read "mg. KOH/100 g. dry wheat."

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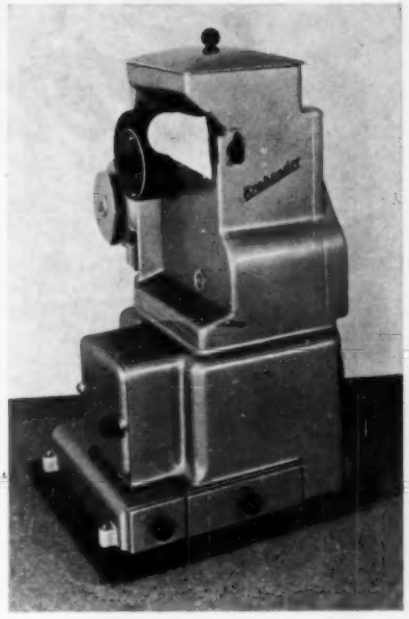

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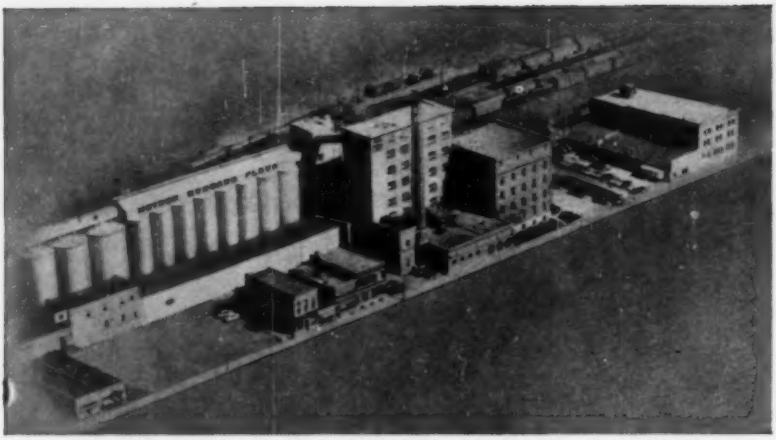

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